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ELECTRON-MICROSCOPIC STUDIES OF RECURRENT HYPOPYON UVEITIS

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Introduction

The histopathological changes in recurrent hypopyon uveitis have been studied by several investigators; Oguchi, Ishiguro, Ueoka, Yoshida, Martin and Sezer, and considerable data has been presented by them, but the fine structure in greater detail still remains to be fully clarified. The authors attempted to investigate some of the pathological changes under electron microscope of an eyeball removed from a case of recurrent hypopyon uveitis.

Report of case

M.M., aged 26, was admitted to our hospital on January 15, 1956. Inflammation had started in his left eye 20 months previously, and his vision was gradually failing. Recently the patient had started to complain of his right eye, and vision was counting figures at 4 meters and could not be improved by lenses. The left eye was enucleated because of pain due to secondary glaucoma. Inflammation did not respond to any medical management. No other manifestations of the muco-cutaneo-oculo-uveal syndrome could be found in this patient.

Gross examination

Examination showed considerable hemorrhagic exudate in the vitreous cavity and total retinal detachment to a central strand.

Microscopic examination

There was marked diffuse inflammatory infiltration of the uvea, retina and optic nerve with infiltration of round cells and some plasma cells and eosinophiles.

Cornea. There was a peripheral infiltration.

Sclera. Diffuse and perivascular infiltration could be seen in the anterior portion.

Anterior chamber. The angle was closed.

Iris. There were perivascular infiltration and thickening of the iris with total posterior synechia. Atrophy and destruction of pigment cells were also present.

Ciliary body. Edema and thickening of the ciliary body were present with fairly intensive infiltration and destruction of pigment cells.

Choroid. The choroid showed a diffuse infiltration, not always most intense around blood vesseles, and considerable thickening at several portions. Edema was seen in several vessels of the vascular layer. Chromatophores were not affected.

Lamina vitrea. This could hardly be recognized in many places.

Pigment epithelium. The pigment epithelium was generally edematous, and the pigment content of the cells varied.

Retina. There was total detachment to the central strand. The detached retina was damaged so severely that the usual pattern of layers could not be recognized.

Optic nerve. The disc showed intense inflammation, which had extended through the area cribriformis into the stroma. In some areas many vacuoles and proliferated glial cells could be seen, and the former resembled Schnabel's cavity produced by atrophic processes. Most vessel walls were thickened, while some were obliterated.

Electronmicroscopic Observation

Materials and Methods

For electronmicroscopy, small pieces of optic nerve near the disc, and pigment epithelium and choroid in the equatorial area were excised, fixed for 3 hours in a one percent solution of osmium tetroxide in phosphate buffer of pH 7.4 and embedded in butyl methacrylate and methyl methacrylate (7 : 3) at 45°C.

Sections obtained with a Japan microtome were examined in a Hitachi Electron Microscope type HU-10 and HS-6.

Observations

Pigment epithelium. The lateral cell surface was smoothly delineated and its distal parts showed a terminal bar structure. At the free surface some of the microvilli-like processes were missing, shortened, swollen or opened. Occasionally the basal cell border exhibited slight destruction. The ovoid shaped nuclei were not affected. A little endoplasmic reticulum seemed to be arranged in the apical region of each cell in a vesicular form. Some had elongated profiles, but did not have Palade' granules.

The initial impression obtained was that the cytoplasm was filled with numerous vacuoles and a few mitochondria of various sizes and forms associated with electron dense Fuscin's granules (spindle and ovoid shaped). The profiles of mitochondria were scattered through the basal region of each cytoplasm, and vacuoles at random through the apical region. Only a few mitochondria had an obvious internal structure consisting of closely paralled cristae, but the greater majority had elongated profiles as well as disappearance of the internal structure in varying degrees.

Simultaneously enlarged electron dense inclusions could be seen at the peripheral region of the elongated mitochondria or vacuoles. Several spindle shaped inclusions (Fuscin's granules) seemed to have broken out of vacuoles. The number of Fuscin's granules in each cell varied considerably.

Basement membrane. At lower magnifications, this was recognized as a continuous linear membrane, but at a higher magnification, marked irregularity in thickness and many discontinuous points could be discerned.

Elastic membrane. There was not only intensive destruction but also irregular expansion of the interspace between the two membranes, in which lymphocytes, neutrophiles and collagen could be recognized. In another portion of the membranes, the micrograph showed that the destroyed membranes are entirely replaced by prolife-

rated collagen.

Choroid. In the choroid there was slight cell infiltration with lymphocytes, neutrophiles and eosinophiles. The endothelium of a vein, adjacent to the elastic membrane, was partially missing. In several veins in the vascular layer, slight degenerative changes of the endothelium (vacuoles and partial missing), destruction of the basement membrane and pericytes, and proliferative penetration of collagen could be seen. Chromatophores showed no changes.

Optic nerve. In the optic nerve a little behind the area cribriformis, the myelin sheaths and axones showed extensive destruction and in some regions they were totally missing, and replaced by proliferated collagen. In some cross sections of destroyed nerve fibers, the lamellar structure of the myelin sheath could hardly be recognized. Even the remaining axonfilaments had a granular appearance and irregular arrangement. These findings probably mark a critical point in the disappearance of axonfilaments. Mitochondria are hardly recognizable in the axonplasm. The sheaths of bundles of nerve fibers were irregular in thickness or generally destroyed.

The relatively abundant collagen and fragments of destroyed nerve fibers were features peculiar to this degenerated optic nerve.

Finally in the walls of veins in the optic nerve, there were destruction and disappearance of endothelial cells, basement membrane and pericytes, associated with many intracytoplasmic vacuoles. Occasionally the lumen of the veins was partially occluded with destroyed matter.

Discussion

The histopathological changes of recurrent hypopyon uveitis described above are generally in accord with those of several investigators. Non-specific diffuse infiltration consisting chiefly of round cells, plasma cells, monocytes and neutrophiles was found in the retina, uvea and optic nerve. Another histologic characteristic was perivascular infiltration, considered to be allergic, without any periateritis nodosa.

The greater resolving power of the electron microscope has now demonstrated the pathological changes more clearly in the pigment epithelium, choroid and optic nerve.

Pigment epithelium: The report of Yamada and Iwaki, is the only one available on the ultrastructure of normal human pigment epithelium. The presence of short or swollen microvilli is believed, as has been suggested by Ikui, to be due to the retinal detachment which had been present for a long time. In 1957, Iwaki reported that endoplasmic reticulum is, for the most part, composed of smooth surfaced endoplasmic reticulum. However, in this case, it was rarely encountered in the vesicular form only, but was found growing to various sizes. Mitochondrial profiles were irregular in form and size, presumably in transition form to larger vacuoles, containing various densities and lying close to the cell surface. All these findings are similar to the

degenerative changes of mitochondria observed experimentally by Rhodin, Gansler, Rouiller, and Sakaguchi in the epithelium of glomeruli and nephritic ducts, by Onoe et al. in human liver cells in CCl_4-toxicosis, add by Tsujimura et al. in liver cells of mice with D.A.B.-cancer. Consequently it may be said that the presence of degenerated mitochondria and their transformation to various vacuoles, and yet a diminution of endoplasmic reticulum,—all these can be recognized as degenerative changes of pigment epithelium. Golgi's apparatus, Lipocrin, or myeloid bodies were not observed. The electron dense, spindle or ovoid shaped components (Fuscin) were scattered in the field of the cytoplasm, but their number varied widely among the cells. Some in prophase lay inside larger vacuoles or inside smaller vesicles. These findings seem to permit the suggestion that Fuscin's granules probably develop from endoplasmic reticulum and mitochondria, but this fact can not be determined because of the presence of an inflammatory process in the eyeball. This concept is supported by the findings described by Õ, who studied the thyroid gland and observed that endoplasmic reticulum might very possibly be transformed into secretory granules.

The basement membrane and elastic membrane

In higher magnifications, the electronmicrograph showed intense destruction of the membranes, sometimes replaced by proliferated collagen, associated with irregular expansion of the interspace and cellular infiltration between them. These findings are generally in accord with optical observations.

Choroid

Pathological changes observed in the choroid, were cellular infiltration and slight destruction of veins, partial disappearance of endothelyum, destruction of the basement membrane and pericytes, proliferative penetration of collagen into the walls of vessels and the appearance of vacuoles in the endothelium. The vacuoles might have been artefacts, but the same finding was also observed by Trier, who studied the capillary endothelium of the thyroid gland of the monkey. Consequently all these findings are probably due to the inflammatory process, although optical changes were hard to recognize.

Optic nerve

A little behind the area cribriformis, the optic nerve showed intense destruction, so that normal features of the myelin sheath and axon could not be recognized and in some areas they were replaced by proliferated collagen. Neither the lamellar structure of the myelin sheath, nor normal profiles of mitochondria could be seen. In 1958, Robertson reported that in the frog's sciatic myelinated fibers soaked in hypotonic or hypertonic solutions, early changes were seen, which involved distinct splitting along the intraperiod line, and the swelling and fragmentation of myelin associated with shrinkage of the axon. In 1958, Ogura, who studied the effect of the ionic concentration of the immersion medium on the structure of peripheral nerves, found that after prolonged treatment with hypertonic solution, there were observed, shortening

Fig. 1. The micrograph shows a relatively large field of cytoplasm in a pigment epithelial cell, containing Fuscin's granules (P), mitochondria (m), and many vacuoles (v). Vesicles (vs), terminal bar (t), lateral cell membrane (lm), microvilli(mv), basement membrane (B). ×7500

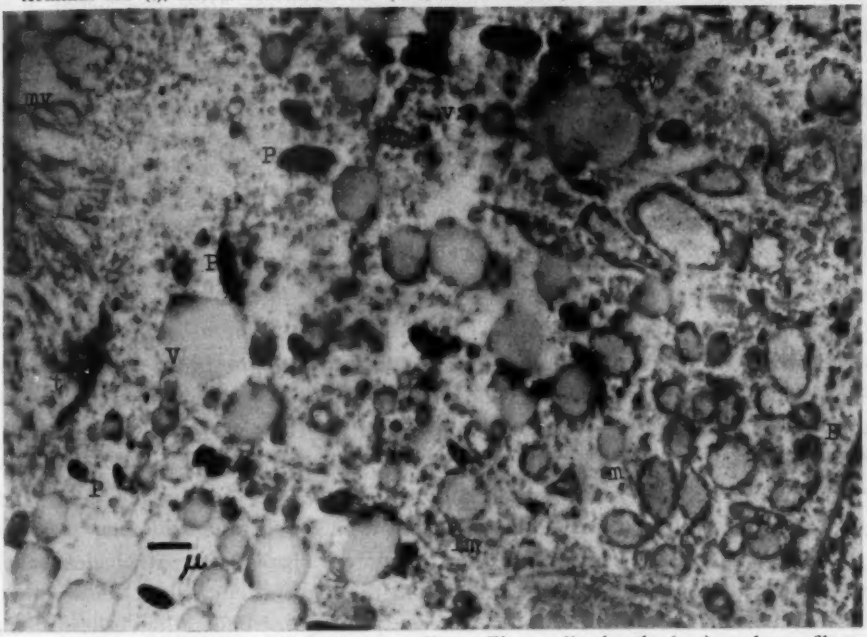
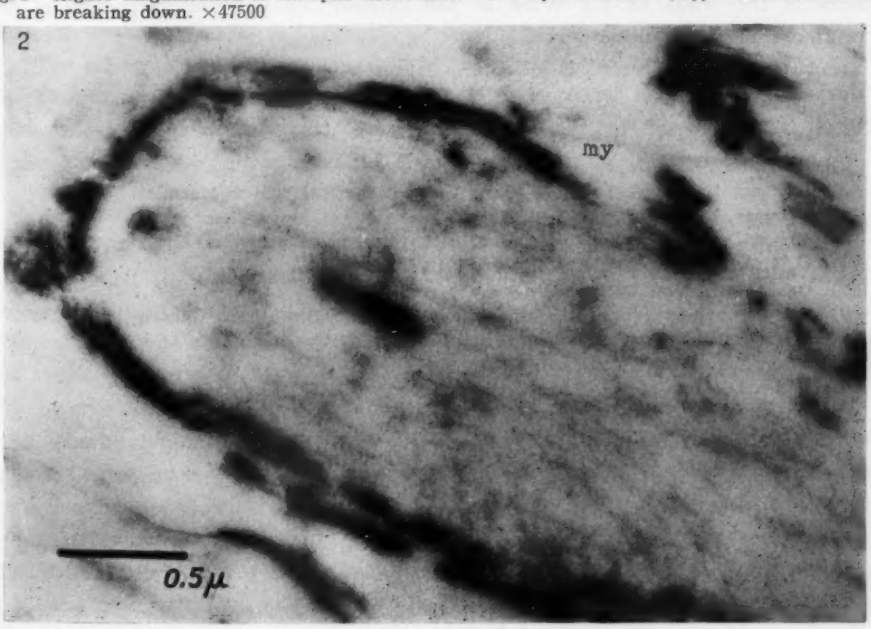


Fig. 2- Higher magnification of the optic nerve fiber. The myelin sheath (my) and axonfilaments are breaking down. ×47500



of axonfilaments and sometimes granular appearances as early changes. In 1957, Nishiura described similar degenerative changes resulting from destruction of the myelin sheath and axon in lepromatous peripheral nerves.

Based upon the observations by these investigators, it is concluded at the present time that degenerative changes of myelinated nerve fibers are indicated by shrinkage of axons, disappearance of axonfilaments, and swelling and fragmentation of myelin sheaths. Consequently the results obtained may be assumed to be the last stage of degeneration of the optic nerve.

Summary

By means of electronmicroscopic observation, the authors could observe in detail the pathological changes of the retina, choroid and optic nerve of an eyeball removed from a case of recurrent hypopyon uveitis, which proved to be a very satisfactory and interesting method for observing the tissue, though the problem of artefacts needs further precise observation. No virus-like particles could be detected in any of the thin sections observed.

The histological changes were as follows. There was non-specific diffuse infiltration consisting of round cells chiefly in the retina, uvea and optic nerve, without any periarteritis nodosa to be found. Electronmicroscopically, the pathological changes were demonstrated by degenerative processes in the pigment epithelium, destruction of the basement membrane and elastic membrane, presence of infiltrating cells and slight changes in the walls of the veins of the choroid, and intense destruction of nerve fibers and veins in the optic nerve.

(Published in J. Clin. Ophth. 13:393, 1959 in Japanese.)

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MORPHOLOGICAL STUDIES BY ELECTRON MICROSCOPE ON THE CILIARY ZONULE

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In spite of the numerous reports hitherto on the structure of the ciliary zonule no definite conclusions have been reached. This may be attributed to its frailty in the structure. Up till 1880, in regard to the structure of the zonule, it was generally believed that the structure was in the nature of a membrane. Since then and up to the present Czermak and Beger's fiber structure theory has been generally accepted. However, as to its details very little is known. It was noted that there are very few electron microscopic studies on the zonule. A review of literature reveals the following 2 reports. According to Ruska and Schwarz the corresponding fiber in cattle eyes has a width of 50-400 mμ, closely resembles collagen fiber and ash an encircling cycle of 77 mμ. Binder and Orth reported the presence of slanting stripes on human zonule fiber with a minimum width of 10 mμ. Thus, the present author has conducted studies on rabbit, cattle and human eyes and has presented her findings here.

Materials and Methods

As material, fresh rabbit, cattle and human eyes were used and processed in an identical manner as follows.

1) The ciliary zonule was removed from the eyeball and crushed without fixing.

2) The material was fixed with osmic acid and crushed. Further, in the rabbit eye only, experiments were conducted with chymotrypsin produced by the Mochida Pharmaceutical Company. However, the results were not too different from the other.

Results

It was found that the width of the fiber in the rabbit eye ranged from 33.3 mμ to 666 mμ with an average of 470 mμ. In the cattle eye the corresponding figures are from 40 mμ to 666 mμ with an average of 515 mμ. In the human eye the corresponding figures are from 166 mμ to 291 mμ with an average of 217 mμ.

As regards structure, in the rabbit eye, while not showing neat cycles as in collagen fiber, a slanting cycle was seen. These fibrils were corded together forming a tightly entwined double corkscrew (Fig. 1, Fig. 2). The width of this cycle ranged from 208 mμ to 500 mμ. Further, it was noted, that the fibers with this cycle were invariably over 300 mμ.

In cattle eye, while the structural aspects were not as neat as in the rabbit eye, it was noted, that a somewhat similar slanting cycle was present (Fig. 3,

Fig. 1. Rabbit eye. ×12,000

Fig. 2. Rbbit eye. ×12,000

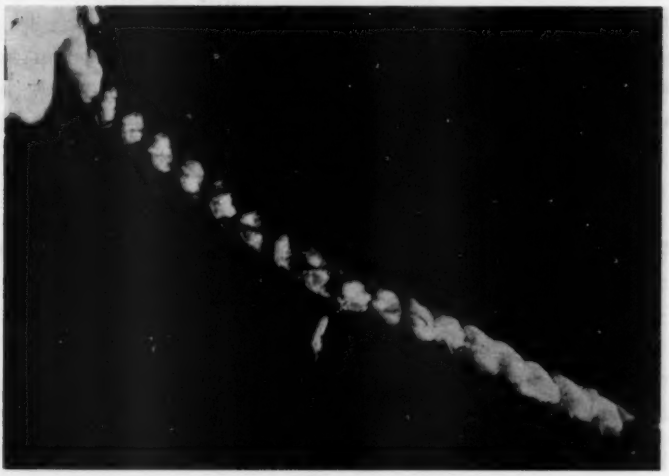
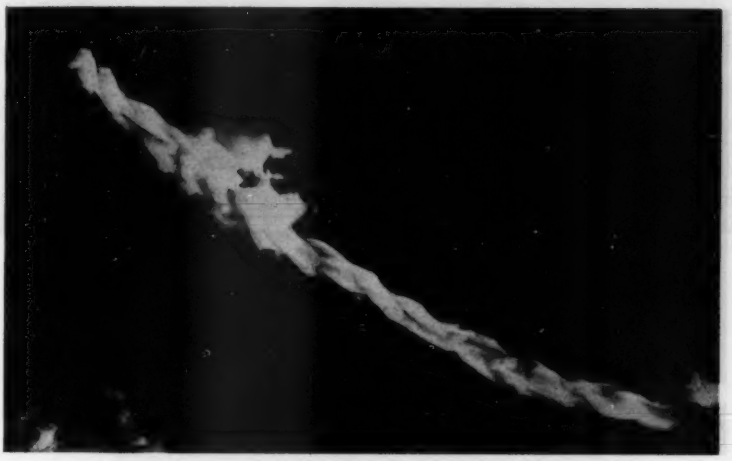


Fig. 3. Bovive eye, ×11, 500

Fig. 4). The width of the cycle in question ranged from 140 mμ to 460 mμ with an average of 330 mμ. The fibers with cycles, as in the case of rabbit eye, were found to be invariably over 300 mμ.

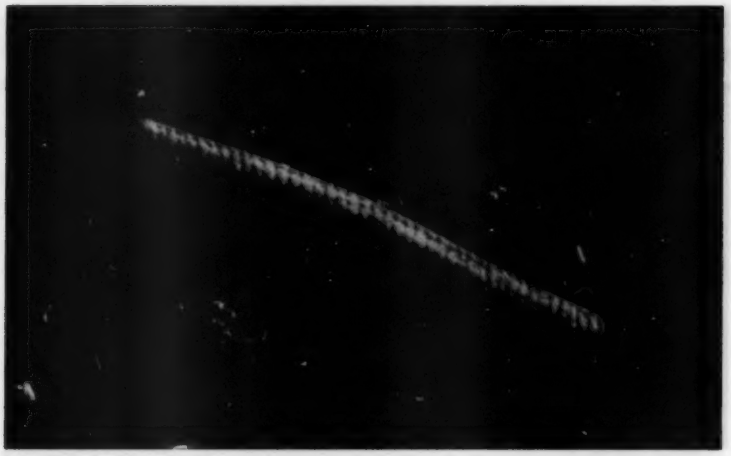
In the human eye, it was revealed, that neat slanting cycles as seen in collagen

Fig. 4. Bovive eye. ×12,000

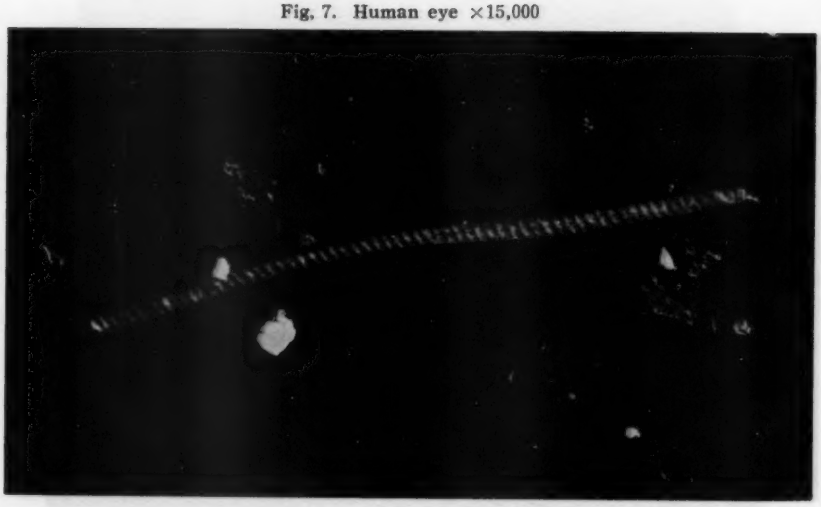


Fig, 5. Human eye ×18,000

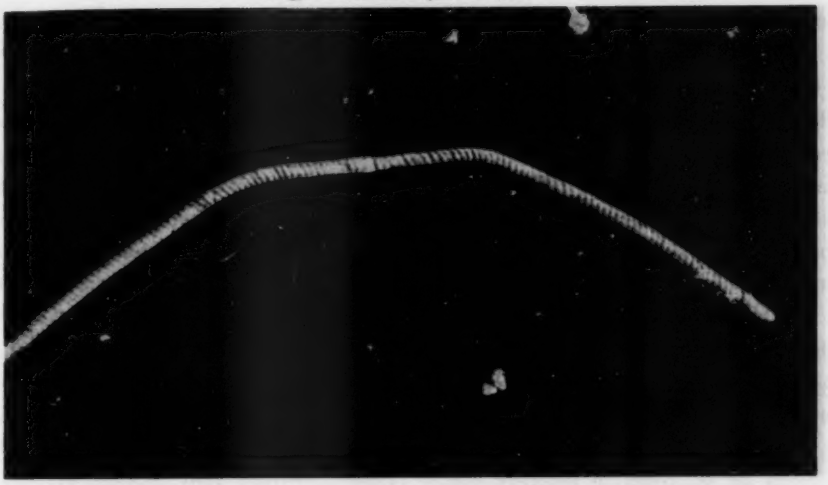
Fig, 6. Human eye. ×18,000



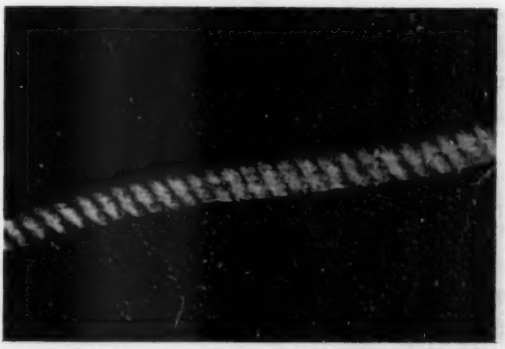
Fig, 7. Human eye ×15,000



Fig, 8. Human eye. ×15,000



Fig, 9. Human eye, ×30,000



fiber were present(Fig. 5, Fig. 6, Fig. 7, Fig. 8, Fig. 9). The width of the cycle showed an average of 66 mμ. It was found that the slanting cycle showed a neat arrangement to the end of the fibers. No smender fibers were found in which cycles were not seen.

In regard to the length of the fiber, in spite of the possible severing of fibers at the time of crushing the longest fiber seen was in the rabbit eye 8.4 μ (Fig. 2), in the cattle eye 8.3 μ (Fig. 4) and in the human eye 13 μ (Fig. 8). Further, in the human eye it was noted, that the thicker fibers were shorter and that the slender fibers were longer. In the rabbit eye and cattle eye a slender fiber under 300 mμ, with no cycle which may possibly have become unified was seen. Among such slender fibers, a slender fiber with no cycle which may possibly have become detached from a bundle of

fibers which have a slanting cycle was seen (Fig. 10).

In addition, among these slender fibers, fibers with sharp ends were observed. This may coincide with the findings of W. Böke and E. Lindner who reported fibers with one end or both ends which were sharp in the cattle's eye ciliary zonule.

Hitherto, the state of bandage among ciliary zonule fibers has been unknown. Whereas, Salzmann, Lauber et al, suggested a complete fusion theory, Shultze advocated a bandage by viscos substance and Beavieux said, that a membrane like bandage was plausible. These however are merely based on optical microscopic observations. In the present experiments the author observed many cases in which fibers with slanting cycles were enclosed in a membranelike substance in the rabbit's eye and cattle eye (Fig. 1 rabbit eye, Fig. 3 cattle eye). In addition it was observed, that the individual membrane like substances were connected together.

Now, it is still unknown whether ciliary zonule fibers are collagen fibers or elastic fibers. However, in 1949 J. Gross made his report based on electron microscopic studies. Until then, elastic fibers were considered morphologically as a sub type and were considered to be close to collagen fibers. Gross reported that 2 filaments entwined and formed a corkscrew like thread, clearly unlike collagen fibers. He reported, that when the filaments become separated no cycles are seen.

Summary

Based on this, in so far as the present author's experiments are concerned, it is conjectured, that the ciliary zonule fibers are close to elastic fibers. The author is of the opinion that these slender fibers have entwined and formed a corkscrew like formation which are separately covered by a membranelike substance, which combined or bonded together, form the ciliary zonule.

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STUDIES ON THE CELLULAR LESIONS INDUCED BY ADENOVIRUS TYPE 8 IN TISSUE CULTURE AND ON THOSE OF THE CONJUNCTIVAL EPITHELIUM OF EPIDEMIC KERATOCONJUNCTIVITIS

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There are many reports about the cytological studies on the cojunctival epithelial cells of epidemic Keratoconjunctivitis, but the essential changes are still now obscure, because primary and secondly lesions are mixed up in these descriptions.

Recently it has been proved that adenovirus type 8 might be the causative agent of epidemic Keratoconjunctivitis, but there are only one or two brief reports about changes of cells infected with this virus.

The present work was undertaken to study cellular alterations induced by the adenovirus type 8 in the human conjunctival epithelium and HeLa cells in tissue culture and to compare these with those of the conjunctival epithelium of epidemic Keratoconjunctivis.

Materials and Method

1. Cultivation of human conjunctival epithelial cells

a) Human conjunctival tissue surgically obtained was cut into fragments about 1×1 mm and centered in a drop of chicken plasma wnich had been previously placed on a cover slip, then chickembryo extract was dropped on the preparation. After the plasma coagulates, the preparations were inverted in the test tube which contained nutrient fluid and incubated at 36.5°C. The growth medium was YLE solution plus 5% human serum and 10% calf serum. When the cell sheets were formed around the primary explants, the supermatant medium was removed, the test tubes were washed twice with Hanks solution, replaced with a fresh medium containg virus inoculum and incubated further at 36.5°C until cytopathogenic changes were observed. The maintenance medium was YLE solution plus 10% chicken serum. Adenovirus type 8 was the prototype strain which was send from the National Institute of Health, U.S.A. and held in the Institute for Infectious Diseases, University of Tokyo.

b) Trypsinized human conjunctival epithelial cells (Chang's strain) were grown at 36.5°C on the cover slips which were placed in test tubes. The growth medium was YLE solution plus 5% human serum and 10% calf serum. When the complete

cell sheet was formed over the cover slips, the supernatant medium was removed, the tubes were washed twice with Hanks solution, replaced with fresh medium containing virus inoculum and incubated further at 36.5°C until cytopathogenic changes were observed. The maintenance medium was YLE solution plus 10 % chicken serum. The virus studied was the same as above mentioned.

2. Cultivation of HeLa cells

Preparations of HeLa cells infected with adenovirus type 8 were made in the same way as Chang's conjunctival epithelial cells. Growth medium was YLE solution plus 10 % calf serum. Maintenance medium was YLE solution plus 10 % chicken serum. The cover slips were removed at various periods of time, depending upon the extent of infection as determined by microscopic examination.

Most specimens were stained with Giemsa, Hematoxylin Fosin and Feulgen, following fixation in methylalcohol or Carnoy's fixative.

3. Preparation of conjunctival epithelial specimens of epidemic Keratoconjunctivitis.

The stamp specimens were taken from the surface of conjunctival follicles by pressing with the surface of a slide glass, without anesthesia.

For taking scrapings, the conjunctiva was previously anesthetized and scraped with the edge of a cover slip. Most specimens were stained with Giemsa and Feulgen, following fixation in methylalcohol, absolute alcohol and Carnoy's fixative.

Results

The cellular changes that appeared in the tissue culture coincided with those of the conjunctival epithelial cells of epidemic Keratoconjunctivitis. The changes which occurred mainly in the nucleus were classified into stages 1, 2, 3 and 4, the stages 3 and 4 being further devided into types A, B, and C, as schematically shown in Text figure 1.

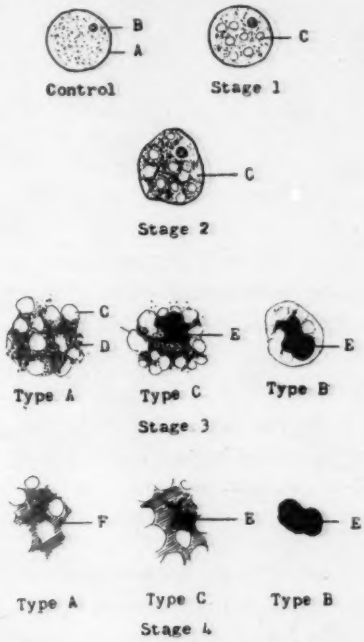
Stage 1: Twenty four hours after infection with this agent, a number of small vacuoles appear within a nucleus. Enlargement of the nucleus and distortion of nuclear shape were not observed, and nucleoli are seen to be normal. These changes can be seen in the conjunctival epithelial cells of epidemic Keratoconjunctivitis on the first day of illness.

Stage 2: Fourty eight hours after infection with this agent, vacuolization of the nucleus becomes more marked, accompanied by redestribution of chromatin.

Some nuclei increase in size, but others not. The Nuclear membrane appears intact but shows a distorted, irregular outline. These changes can be seen in the conjunctival epithelial cells of epidemic Keratoconjunctivitis on the second day of illness.

Stage 3: Seventy two hours after infection with this agent, characteristic alterations appear within a nucleus. The alterations in this stage can be devided into types A, B, and C. The characteristic of type A is a nuclear degeneration with

Text-Fig. 1. The sequence of nuclear alterations in conjunctival epithelial cells of epidemic Keratoconjunctivitis, as determined by comparative studies of tissue culture at varying intervals after adenovirus type 8 inoculation.
(A) nuclear membrane, (B) nuceolus, (C) vacuole, (D) rearranged chromatin, (E) eosino-basophilic mass, (F) karyolysis



more pronounced vacuolization. The nucleus increases in size, being distorted in shape and is without prominent inclusion. The nuclear membranes are destroyed here and there. Type B is characterized by the appearance of eosino-basophilic, Feulgen-positive mass within a nucleus. These eosino-basophilic masses locate in the central or eccentric position in the nuclei and vary in size.

Sometimes the central eosino-basophilic mass has fibrillike connections with the periphery, probably similar to the "honeycomb" structure noted in HeLa cells infected with adenovirus type 3. The eosino-basophilic granules may give a beaded appear ance in the nuclear periphery, or disperse throughout the nucleus besides the mass. Type C is the intermediate form of types A and B. Nuclear degeneration with vacuolization and mass formation can be observed at the same time in a nucleus. The nuclei become swollen and distorted in shape usually. These changes can be seen in the conjunctival epithelial cells of epidemic Keratoconjunctivitis on the second day of illness.

Stage 4 is the most advanced one and is devided into types A, B, and C respectively. In type A the nuclei become markedly shrunk, deeply stained, resembling the

Table 1. The Appearance Rate of the Cellular Lesions of Types B and C in the 3rd Stage in the Conjunctival epithelium of epidemic Keratoconjunctivitis

Day of illnes	Number of cells of types B and C	Number of cells observed*	Percent apperance
1	0	60	0
2	15	550	2.7
3	25	560	4.6
4	23	540	4.3
5	12	520	2.3
6	2	159	1.2
7	2	160	1.2
8	1	190	0.5

* The total of 5 specimens

pycnotic change. Type C is the intermediate form of types A and B. These changes can be observed in the conjunctival epithelial cells of epidemic Keratoconjunctivitis on the second day of illness.

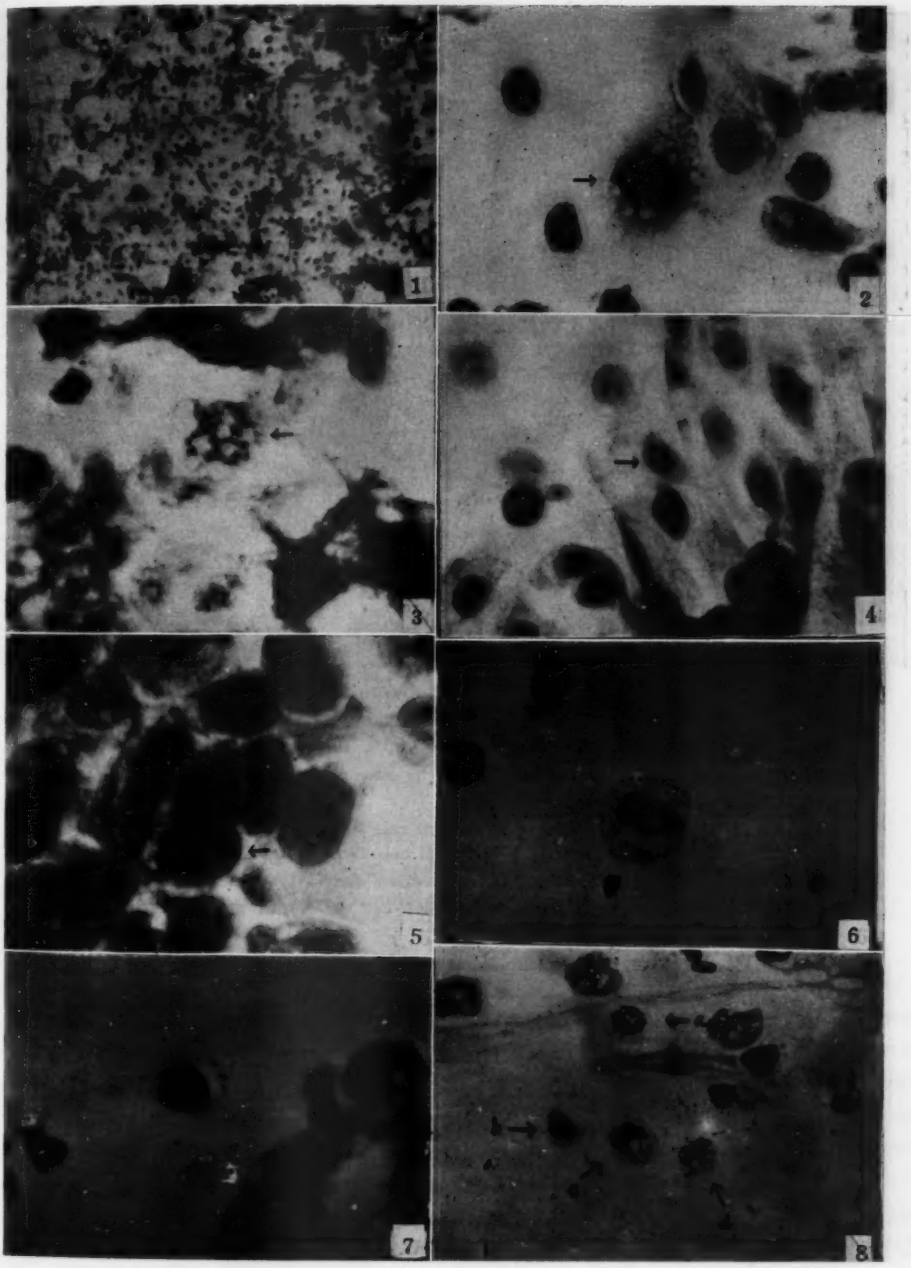
The cellular changes of types B and C in stage 3 which are the most characteristic, appear, as shown in Table 1, on the second day of illness, and can be seen during the first week, but become rarer after the eighth day of illness.

Discussion

The cellular alterations induced by adenoviruses are in the main intranuclear. Recently Prier and Lebeau placed the adenovirus type 2 through 7 into three major groups, i. e. Group 1 (types 3 and 4), Group 2 (type 7), and Group 3 (types 5 and 6). Group 1 is characterized by nuclear enlargement, eosinophilic granulations, and prominent eosinophilic inclusion bodies. Group 2 is characterized by absence of nuclear enlargement. There are, however, finite intranuclear eosinophilic granulations and inclusions resembling those of group 1. Group 3 is characterized by absence of nuclear enlargement. The inclusions here are basophilic.

The characteristic of the cellular alterations induced by the adenovirus type 8 is a vacuolization with or without mass formation in the nucleus. Intranuclear eosinophilic granulations are not observed and masses or inclusions here resmbling th adensvirus type 3 are eosino-basophilic.

Some nuclei increase in size, but others not. These observations indicate, that adenovirus type 8 must be placed in a new position on the basis of the cytologic changes. The cellular alterations induced by adenovirus type 8 coincided with those of the conjunctival epithelial cells of epidemic Keratoconjunctivitis. This supports the virological and serological conclusion, that adenovirus type 8 may be the main causative agent of this disease.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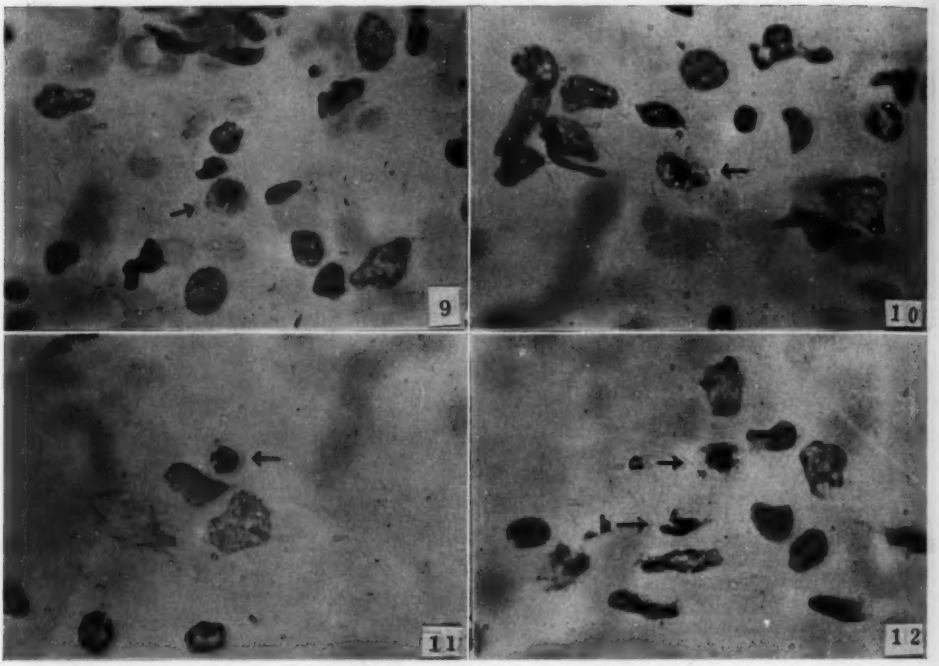

Explanation of figures

Figs 1-5. Cellular alterations induced by adenovirus type 8 in tissue culture, Giemsa staining.

Fig. 1. Human conjunctival epithelial cell 96 hours after infection. ×100

Fig. 2. Human conjunctival epithelial cell 72 hours after infection, stage 2. ×600

Fig. 3. HeLa cell 72 hours after infection, stage3, type A. ×600

Fig. 4. Human conjunctival epithelial cell 96 hours after infection, stage 3, type B. ×400

Fig. 5. HeLa cell 96 hours after infection, stage 3, type B. ×600

Figs 6-12. Conjunctival epithelial cell of epidemic Keratoconjunctivitis. Figs 6 and 7, Giemsa staining, Figs 8-12, Feulgen staining.

Fig. 6. The 5th day of illness, stage 3, type B. ×600

Fig. 7. The 4th day of illness, stage 3, type B. ×600

Fig. 8. The 3rd day illness, stage 3, a, c, d: type C, b: type B. ×400

Fig. 9. The 2nd day of illness, stage 3, type C. ×400

Fig. 10. The 4th day of illness, stage, 3, type B. ×400

Fig. 11. The 3rd day of illness stage 3, type B. ×400

Fig. 12. The 3rd day of illness, stage 3, a: type C, b: type P. ×400

Summary

The cellular changes induced by adenovirus type 8 in tissue culture were investigated. The changes which occur mainly in the nucleus are classified into stages 1, 2, 3, and 4, the stages 3 and 4 being further devided into types A, B, and C. The early change is the formation of vacuoles in the nucleus. It transfers to nuclear degeneration in type A, to eosino-basophilic mass formation in type B and to the intermediate change in type C. The cellular alterations induced by this agent in tissue culture coincide with those of the conjunctival epithelial cells of epidemic Keratocojunctivitis. The changes of types B and C in stage 3 are most frequently seen on the 2-5th day of illness and gradually become rarer after that.

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TRANSPLANTATION OF THE PAROTID DUCT INTO THE CONJUNCTIVAL SAC FOR THE TREATMENT OF XEROPHTHALMIA

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Introduction

A number of methods in the treatment of xerophthalmia had been performed up to now, but the therapy was usually unsatisfactory and gave only temporary improvement.

In 1950, Filatov and his associates first reported the transplantation of the parotid duct into the conjunctival sac for the treatment of total xerophthalmia, and they have reported 80 successful cases[8]. Subsequently Katznelson[6], Ao[1], Yen and Lee[9], Hisia[5] and Lao[7] reported several cases of transplantation of the parotid duct. Recently there is a report of this transplantation by Bennet and Bailey.

In Japan we have had no report of this operation until now. At first we performed experimental work on the dog and human cadaver work was added. The surgical procedure which was devised by these works was applied clinically in 7 cases

of xerophthalmia which occurred following trachoma. The purpose of this operation is to moist the eye with parotid secretion instead of normal tears.

Experiment

Fifteen dogs were used in this work. They were anesthetized with ouropan-soda intramuscularly.

In order to know the position and length of the parotid duct we performed sialography before operation, i.e. a fine lacrimal cannula was inserted into tho parotid duct and injected 0.5-0.6 cc of 40% moriodol into the duct through this cannula. Sialography was taken in the lateral and vertical position.

Surgical Procedure on the Dog

1) A fine whole-bone bougie was inserted into the duct from the os of the parotid duct to aid in the identification of the duct during it's surgical isolation.

2) An area of buccal mucosa 5 mm in diameter surrounding os of the parotid duct was incised and dissected freely.

3) Submucous tissue was undermined superficially toward the parotid gland and then the duct was dissected freely.

4) An incision 2 cm long was made on the lower lid. Subcutaneous tissue was undermined superficirally toward the parotid gland and we created a subcutaneous tunnel.

5) The buccal funnel, attached to the os of the parotid duct, was drawn through this subcutaneous tunnel to the opening on the lower lid.

6) An opening 3 mm in diameter was made on the inferior cul-de-sac at the temporal side and the buccal funnel was drawn into the inferior cul-de-sac through this opening.

7) After removal of the whole-bone bougie from the parotid duct the buccal funnel was placed into the defect on the base of the lower conjunctival cul-de-sac and and sutured.

8) The skin incision on the lid and buccal mucosal defect in the mouth were closed with sutures.

9) Postoperatively the dogs were injected with 300,000 units of penicillin intramuscularlly.

If the parotid duct is too short to reach the inferior cul-de-sac, the incision's area of buccal mucosa must be made wider than usual to form an extention of a parotid duct.

Result

In this experimental work we observed that a copious flow of parotid secretion into the inferior cul-de-sac was produced, especially in the time of feeding. But in spite of an excessive secretion of the parotid duct, the cornea and conjunctiva tolerated it and their appearance was healthy.

The prognosis has been observed over a period of five months without any changes

on the parotid secretion.

Cadaver Work

We learned the anatomical detail of the operative area by means of dissection of a human cadaver to find the best way to transplant the parotid duct into conjunctival sac without leaving the smallest trace on the face as much as we could. Finaly we found that a 2.0 cm buccal skin incision which is shown in Fig. 1. is suitable and enough for the purpose. The position of the skin incision agrees with the anterior margin of the maseter muscle within the limits of the triangle which is made up of three apexs, i.e. ext. auditory meatus, corner of the mouth and lower margin of the nose.

Clinical Application

Sialography

As a preparative procedure for the operation sialoghy was taken in the lateral, vertical and frontal position by the same way as we did in the cases of dogs. (Fig. 2)

Fig. 1.
Comparsion of the positon of buccal skin incision.

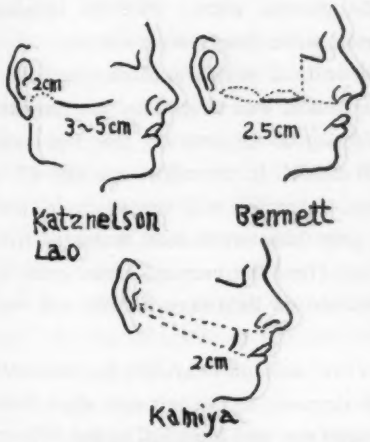


Fig. 2.
Sialogram (lateral position.).

A surgical procedure was as follows:

1) The patient was generalanesthetized.

2) A fine whole-bone bougie was inserted into the duct and let alone till the transplantation came to an end.

3) A 2.0 cm buccal skin incision was made. As described above, the position of this incision agreed with the anterior margin of the masetermuscle.

4) The subcutaneous tissue was undermined and the duct was found deep beneath the buccal fat pad. The isolation of the duct toward the main gland was made carefully, since branches of facial nerve and the transverse facial artery were distributed reticulately in this area.

5) Four surgical silk threads were passed through the mucous membrane about 1.0 cm, far from around the os of the duct.

An area of mucous membrane in the mouth, 5 cm in diameter, around the os of the duct was then incised, pulling up these silk threads. The mucous membrane incision was then carried out through the buccinator muscle and the duct became completely free from its surrounding tissue.

6) A 2.0 cm incision was made on the lower lid, 2.0 mm from the eye lashes. Subcutaneous tissue was undermined superficially towards the duct and the subcutaneous tunnel which communicated with the buccal skin incision was made,

7) Then the duct which acquired a mobility was easily removed to the opening on the lower lid through the subcutaneous tunnel by the aide of a rubber tube 1.0 cm in diameter.

8) An opening was made through the conjuntiva in the lateral one-third of the inferior cul-de-sac and the mucous membrane surrounding the os of the duct was drawn into the inferior cul-de-sac. This mucous membrane was placed on the conjunctival opening and sutured.

9) A whole-bone bougie was removed from the parotid duct. The lid incision, oral mucous membrane defect and buccal skin incision were closed with sutures.

10) Postoperatively the patient was given 600,000 units of penicillin intramuscularlly.

Case 1. T.I., aged fourty years, fairly-nourished man, was first seen on January 31, 1958, with a history of blurring of vision and feeling of dryness in the right eye of about one years' duration. The vision was 20 cm/F.C in the right eye and 0.6 in the left eye. There were trachoma scar and pannus, entropion and trichiasis in both of the eyes. Groups of superficial, slightly raised, grey haze were seen scattered over the corner. The minute superficial vessels, spring from the corneal loops, grew inwards towards the centre. The patient was hospitalized on February 3, 1958 and was taken to the operating room on February 5, 1958.

Case 2. S.K. a woman, aged fifty one years, was first seen on February 18, 1958. with the chief complaint of blurring of vision and feeling of dryness in the left eye since three years ago. The vision was 0.06(0.1×-S.2.0D)in the right eye and 20cm/F.C in the left eye. There were trachomatous scaring and pannus, entropion and trichiasis both of the eyes.

The iris in the left eye was not seen by focal illumination, owing to dense corneal haze. She hospitalized at once and operated on in the left eye on February 19, 1958.

Case 3. U.H., 63-year-old man, visited our clinic on March 13, 1958, with the chief complaint of feeling of dryness in both eyes which lasted in about two years. There was also intense blurring of vision of about three years' duration. The vision was 30 cm/F.C in the right eye and 20 cm/F.C in the left eye. There was a history of trachoma since coildhood and we examined trachomatous scaring and pannus, entropion and trichiasis. The conjunctiva and cornea were completely dry and in a lustreless condition. He was admitted to the hospital, March 24, 1958, and the operation of the

left eye was done on March 26.

The patient was taken to the operating room again for operation of another eye on April 9.

Case 4. F.K., 54-year-old man, was first seen at our clinic on July 12, 1958. He complained of pain and marked epiphora in the left eye since three days ago. In the right eye there was blurring of vision and feeling of dryness of one years' duration. The vision was 5 cm/F.C in the right eye and 0.2 in the left eye with correction. The examination revealed outer sty and corneal ulcer of the left eye. One week later sty and corneal ulcer recovered with suitable therapy, so that he was admitted to the hospital for the treatment of xerophthalmia in the right eye on July 21, 1958. The patient was taken to the operating room on July 23.

Case 5. Y.I., 69-year-old man, was first seen at our clinic on July 21, 1958. He stated that there was the history of trachoma from childhood and less of vision in both eyes since about fifteen years ago. His chief complaint was violent ocular pain owing to dryness of the eyes. The eyes had typical xerophthalmia and were limited extremely in ocular movement due to symblepharon. The vision was zero in both eyes. He was admitted to the hospital on August 2, 1958, to have a transplantation of the parotid duct into the conjunctival sac in the right eye on Augest 6.

Case 6. H.M., 72-year-old woman was first seen on Augest 19, 1958, with chief complaint of feeling of dryness in the both eyes. There was also a blurring of vision of about twenty years' duration. The vision was P.L. in the right eye and 25 cm/F.C. in the left eye. The ocular diagnosis was xerophthalmia and she was admitted to the hospital on Augest 19, 1958.

Case 7. R.K., 54-year-old man, was first seen on Augest 22, 1958. There was a history of trachoma which has been lasting for about fourty five years. He stated that he has been feeling dryness and blurring of vision in both eyes from one month ago. He was admitted to the hospital on Augest 25, 1958 and operated on the right eye on August 27.

In this case the operation procedure was different from other cases, i.e. the process of the transplantation was performed from the opening of the oral mucosa without the use of buccal skin incision.

Postoperative Course and Prognosis

The postoperative course of these cases were substantially identical. Generaly speaking, except for special cases it was as follows:

On the next day after operation, the conjunctival sac was filled up with hemal secretion, which became a clear fluid in two or three days. At the same time there was marked swelling of the operations' area. In a week, when the patients were undressed, they complained that the flow of parotid secretions into the eye was excessive, particularly during eating, which produced marked epiphora. There was more or less facial weakness postoperatively, but this cured completely about in a month.

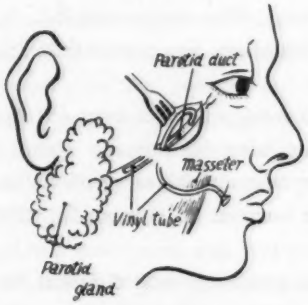
The entropion owing to traction of the inferior cul-de-sac was treated by surgery for entropion. The skin incision healed up with a small linear scar, so that it did not spoil ones' looks. The conjunctival sac and cornea became moist and then gradually the corneal transparency was recovered. We summerized the postoperative course of these seven cases as shown in Table 1.

Table 1.

Case	Age	Eye	Vision before operation	After operation	
				Vision	Lacrimation
1	40	R	20cm/F.C	0.1 13 month	Moderate
2	50	L	20cm/F.C	0.4 14th day	Moderate
3	63	L	20cm/F.C	60 cm/F.C 7 month	Excessive
		R	30cm/F.C	60 cm/F.C 7th day	Stoopage (4 month postoperatively)
4	54	L	50cm/F.C	0.1 6 month	Moderate
5	69	R	0	0 5.5 month	Moderate
6	72	R	P.L	20 cm/F.C 6 month	Decrease gradually
7	54	R	10cm/F.C	20 cm/F.C 2.5 month	Excessivive

F.C: Finger counting H.M: Hand motion
P.L: Perception of light

Fig. 3.



As above mentioned postoperative courses were uneventful, but in three cases we could not have no satisfactory result for parotid secretion. The patient of Case 3 was seen on October 27, 1958, that was about four month after surgery of right eye. He stated that the flow of parotid secretion into the right eye had been decreasind gradually since middle of May and in August the secretion stopped completely, but we could not find out its' cause. The patient of Case 6 complained of formation of a encysted tumor, situated on the lateral site of the lower lid, after stoppage of parotid secretion into the eye in fourty two days after surgery. Therefore, the patient was admitted into the hospital at once and through a skin incision over the encysted tumor, the parotid duct was isolated after removal of it.

Then one end of a vinyl tube 1 mm in diameter was inserted into the parotid duct and the other end was led into the inferior cul-de-sac. The parotid secretion flowed out again into the eye through this vinyl tube.

The patient of Case 7 had been troubled with excess parotid secretion postoperatively. We tried probing of the lacrimonasal duct and building of an artificial lacrimonasal duct which communicated the conjunctiva with the middle meatus by use of an epidermal tube[2], but these were only partially successful. Therefore, as showed in Fig. 3, a vinyl tube 2 mm in diameter was inserted into the parotid duct and the other end led into the mouth beneath of buccal skin. In this way the parotid secretion flowed out into the eye as well as into the mouth.

This method was effective to decrease lacrimation, but a cyst was formed along the vinyl tube. At last we had to transplant the parotid duct into it's original position.

Comment

In all seven cases the cornea got it's transparency, gradually back, subsequently all patiens increased visual acuity, and also the unsound condition of the conjunctiva was improved. As causes of these findings it might be considered, that the parotid secretion gave some nutrition to the cornea and conjunctiva in addition to moistening them.

From the result of these cases we are able to recommend our operation for treatment of xerophthalmia as the best method. But three unsatisfactory cases presented themselves after observation for several months. The causes for them were stoppage, decrease and excess of secreting.

The causes of decrease and stoppage of secretion were considered to be construction of surrounding tissue on the conjunction between the os of the duct and mucous membrane which was used to extend the duct, and in one case, a cyst was performed on this part. In a case which suffered from prominent excessive secretion, there was no help it in spite of every possible endeavor.

On account of plastic culture of the face after operation, only a small scar remained which did not spoil one's locks. In Case 7, we succeeded to transplant the parotid duct without buccal skin incision, so that, in future, transplantation of parotid duct should be performed without buccal skin incision.

Summary

We confirmed the feasibility of the transplantation of the parotid duct into the conjunctival sac by experimental work on the dog and human cadaver work. The parotid duct has been successfully transplanted into the conjunctival sac in fifteen dogs. Our procedure was applied clinically in 7 cases of xerophthalmia which occured following trachoma.

The prognosis of these patients were observed postoperatively for 2.5-13 months. We had successful treatment of for cases. And we had also unsatisfactory result in

three cases, caused by stoppage of secretion, formation of cyst and excessive secretion.

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STUDIES UPON THE CILIARY BODY

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Introduction

The instruments and method which hitherto have been deviced to observe the region of the ciliary body are nothing but those based on the principle of the cystoscope, for example Kajiura's cycloscope or Kamiyama's oculoscope. We can easily understand that such stereotyped experimental measures should have serious defects, that during the observation the intraocular pressure through the loss of the vitreous ought to be lowered and the results obtained deviate from the normal.

Considering from such standpoint, the author thought out an unique idea based upon the principle of ordinary ophthalmoscopy, namely, when a mirror is enclosed in the vitreous the inside view of the anterior uvea ought to be imaged upon this mirror and then we can easily observe this reflected image by way of common ophthalmoscopy.

Practically applying this original idea the author succeeded to observe continuously the region of the ciliary body and the backside of the iris at longest during one week under normal intraocular pressure.

Experimental Method

A plane mirror (20 mm.×3 mm.×1 mm.) was used to insert into the vitreous. To keep the mirror clear I used also another kind of mirror enclosed its backside with silver plate and sometimes used a similar one with a small hole in its margin to suture it with the sclera. The most important part of the inserting procedure is, after local or general anesthesia and exposure of the upper part of the sclera, to kauterize the sclera with ultrashortwave diathermy, for purpose of preventing the bleeding from the incised wound, which is situated 3-5 mm. upper from the corneal limbus, incised

5-6 mm. horizontally. The incision of the sclera is made with previous sutures pulling them up to avoid the prolapsus of the vitreous, but following the scleral incision and the insertion of the mirror, some amount of vitreous outflow was inevitable and the intraocular pressure lowered to some extent, for example it was 25 mm. Hg. before the operation and 6 mm. 30 minutes after the operation, 8 mm. after one hour, 9 mm. after 90 minutes. When it was necessary to restore the pressure rapidly after the operation, the injection of 0,3-0,5 cc. physiological saline solution into the vitreous was able to raise the pressure to 18 mm..

As experimental animals I used at first 14 eyes of albino rabbits and then 3 eyes of dogs, which obliged to operate with general anesthesia.

The observation of the mirror image is possible instantly after the inserting operation, however postulating some skill and experience, and almost all cases show a clear image during 24 hours. Twenty four hours after the mirror insertion slight dusty infiltration is found in the anterior chamber but such infiltration does not hinder the ophthalmoscopy. The most regrettable obstacle is, that the mirror surface soon becomes unclear owing to the detachment of its backside silvernitrate membrane.

Results

Normal findings of the ciliary body

The author could clearly distinguish the pupillary margin, the backside of the iris, the equator of the crystalline lens, the ciliary vascular plexus, afferent and efferent vessels, the vortical veins. The view of the dog eyes was darker than that of the rabbit eye and its details were more difficult to distinguish.

Observation on the apperarance of intravasculary injected pigments in the region of the ciliary body.

In the purpose to clarify the productive mechanism of aqueus humor, transudation or excretion of various agents and pigments into the anterior chamber has been investigated by many authors. But up to now, a direct method to observe the ciliary body has not yet been invented. The author investigated the outflow state of the pigment from the vascular plexus of the ciliary body, inserting a mirror into the

Table 1. Results of Intracarotid Injections Condition

	Condition	Colorization of ciliary vessels	Outflow of the pigment into the posterior chamber
Indigo-carmine	normal ocular pressure	(++)	(+)
Fluores-ceine	normal ocular pressure	(++)	(+++)
	high ocular pressure	(—)	(—)
	ciliary dody kauterization	(+)	(+)

vitreous of the rabbit eye, observing the image of the ciliary body on the mirror from outside of the eye with the common ophthalmoscope and injecting 1% indigocarmine or 5% fluoresceine into the carotid.

1% indigocarmine: instantly after the injection into the carotid some of the afferent and efferent vessels rapidly dilate and then color blue. The region of the vascular plexus colors and discolors most slowly. The outflow of the pigment into the posterior chamber occurs slowly as if smoke curls up, and neither pulsatorily nor rythmically as other authors reported.

5% fluoresceine: almost simultaneous with the injection the posterior chamber colors suddenly dark yellow showing no dilatation of the ciliary vessels and after thirty minutes still remains of slight color in the posterior chamber.

When the intraocular pressure is artificially heightened neither the outflow of the fluoresceine into the posterior chamber nor the colorizatin of the vessels are observed. And when the ciliary is previously kauterized by diathermy just like the operation of the glaucoma, the body outflow of the fluoresceine into the posterior chamber is markedly inhibited.

Table 2. Results of Intravenous Injections

	Colorization of ciliary vessels	Outflow of the pigment into the posterior chamber
Indigocarmine	+	−
Fluoresceine	+	+
Methylenblue	+	−

When 1% indigocarmine, 5% fluoresceine or 1% methylenblue is injected into the auricular vein of the albino rabbit, the states of the appearance of the pigment in the ciliary vessels and in the posterior chamber are shown in Table 2.

The influence of various autonomous nerve poisons on the ciliary vessels

1) 0.1 cc. of 1% atropine injected subconjunctivally:

after 15 minutes showed marked dilatation of the ciliary vessels and after 50 minutes the dilatation and congestion were decreased but there still remained some dilatation in the vascular plexus region.

2) 0.1% epirenamine:

after 5 minutes a striking constriction of the total vessels appeared, but after 50 minutes dilatation and tortuosity just like a loopshaped figure could be seen.

3) 0.5% eserine:

after 10 minutes afferent and efferent vessels dilate and become tortuous but after 60 minutes the vessels show constriction and anemia.

4) 1% pilocarpine:

after 15 minutes afferent and efferent vessels dilate irregular and after 40 minutes show diffuse congestion.

Summary

The author deviced an original method to observe the region of the ciliary body, namely inserting a plane mirror into the vitreous behind the crystalline lens, suturing up the operated wound and after the intraocular is restored, observed the image on the mirror by way of common ophthalmoscopy. The observation was possible to be continued at least 24 hours and at the longest one week.

Applying this technique the author investigated the state of outflow of several pigments which were injected intracarotidly or intravenously. Among other things, when the indigocarmine was injected in the carotid the outflow was not pulsatorily or rythmically but slow smokelike. This difference between the results may depend the condition of the ocular tension, because the previous reports were made in the condition of lowered tension and when the ocular tension was hightened artificially no pigment outflow was observed.

The influences of various autonomous nerve poisons on the ciliary vessels were also investigated, when the agent was subconjunctivally injected and the changes of the ciliary vessels followed up during one hour. Atropine produced in early stadium intense dilatation and congestion but in the late stadium gradual contraction afterwards. Eserine produced at first marked dilatation and congestion but contraction and anemia after one hour. Pilocarpine produced at first irregular dilatation and afterward diffuse congestion of the vascular plexus and the vessels of the iris.

(Published in Acta O. Jap. 61:201, 1957, 63:155, 1959 in Japanese.)

Fig. 1. Method of observation.
M: mirror in the vitreous
O: ophthalmoscope

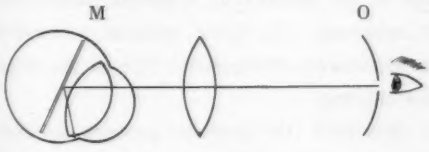
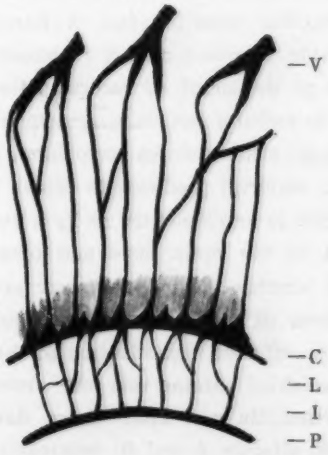


Fig. 2. Normal finding.
P: pupillary margin
I: backside of the iris
L: equator of the lens
C: ciliary vascular plexus
V: vortial veins



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PATHOHISTOLOGIC STUDIES ON THE DAMAGED ARACHNOID AND OPTIC NERVE OF THE RABBIT INOCULATED WITH ANTIRABIC VACCINE INTO THE CISTERNA MAGNA

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In view of experimental evidence it is now agreed among many investigators that quadriplegia and optic nerve damage resulting from antirabic vaccination are due to allergic reaction caused by the brain material contained in the vaccine. Experimental approach to clarification of the true nature of postvaccinatory retrobulbar neuritis, however, has so far been attempted only by Uchimura et al.

In his about 300 craniotomized and some autopsied cases of retrobulbar neuritis Imachi nearly always found remarkable thickening, turbidity and adhesion of the arachnoid especially around the optic chiasm, and on various occasions he expressed his view that mechanical oppression by the pathologic arachnoid, and disturbance of the intracranial fluid resulting therefrom would exert a baneful influence upon the optic nerve.

In medical literature meningitic neuritis is reckoned as one of the chief causes of retrobulbar neuritis, but in Europe there are some scholars who hold demyelinating multiple sclerosis mainly responsible, if not exclusively, for retrobulbar neuritis; they even go the length of asserting that this disease in the absence of systemic demyelination is nothing but monosymptomatic multiple sclerosis. In their opinion arachnoid damage observable in retrobulbar neuritis is a secondary development due to the phlogistic material produced to repair the sclerosing lesions.

But is arachnoiditis really a result of optic neuritis? Or dose it precede involvement of the optic neve and other meninges? Or dose it occur simultaneously with optic neuritis through the same process? Experimental methods alone can solve this problem of the causative mechanism of arachnoiditis and retrobulbar neuritis. The author effected sensitization of the meninges and the optic nerve by direct inoculation of antirabic vaccine into the cisterna magna, and investigated the relation between arachnoiditis and optic nerve damage pathohistologically. It was also studied what effects vitamin A and B_1 deficiencies would have upon development of the lesions.

Method

Normal male albino rabbits weighing 2 kg were divided into three groups, and respectively fed on normal, vitamin A and B_1 deficiency diets. They were the injected with the antirabic vaccine prepared by the Hyogo Higienic Laboratory in 0.3 cc quantities into the cisterna magna. Reinjection of the vaccine was conducted after an interval of two weeks. All the animals were decapitated in the period between a few hours and 10 weeks after injection. Specimens were stained with Marchi, hematoxylin-eosin, Van Gieson and Sugamo myelin sheath stains.

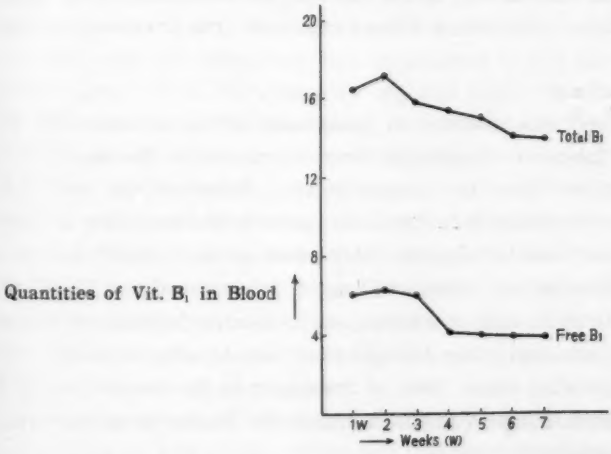
The normal diet per day consisted of 300 gr of bean curd refuse with some vegetables added. The animals were put to experimental use after two-week feeding. The feed was continued during the experiments.

The vitamin A deficiency diet consisted of the same amount of bean curd refuse exoleated with a Soxhlet's apparatus, and then water added and 0.5 gr of vitamin A-lackin Panvitan powder. Vitamin A concentrations in blood were measured after the G-D-H method during the preexperimental period. Also general condition and changes in bodily weight were carefully noted. The animals were put to experimental use after 10 days of this feeding. Though they developed no clinical symptoms, they

Table 1. Quanties of Vitamin B_1 in Feed

	Normal diet (γ %)	Vitamin B_1 Deficiency Diet (γ %)
I	84.0	37.2
II	87.5	32.5
III	70.0	31.0
mean	80.5	33.5

Fig 1. Vitamin B_1 concentration in rabbits feeding on vitamin B_1 deficiency diet B_1 concentration in blood.



showed statistically significant decrease of vitamin A concentration in the blood, compared with the normally-fed.

Vitamin B_1 deficiency diet consisted of the same amount of bean curd refuse processed in an autoclave by heating at 120°C for 30 minutes under 2 atmospheric pressures (Table I). Vitamin B_1 concentrations in blood were determined, using thiochrome. The animals were experimentally used only after they had been ascertained to be short of this vitamin (Fig. 1).

Results

Normal Diet Group

Slight inflammation of the arachnoid was noted in the immediate period after the the first injection. The inflammatory cell reaction was so mild that proliferative changes did not occur. Reinjection, however, produced a very conspicuous tissue reaction, and this allergic inflammation did not subside so easily.

After the first injection a small number of Marchi's balls were observable in the optic nerve during a certain period, but this appearance of the balls was brought on by direct action of the vaccine, and seemed to have nothing to do with arachnoiditis. Four of five weeks after injection these balls were already gone from the optic nerve. Reinjection, however, produced marked degeneration of the optic nerve, and 21 days later Marchi's balls were demonstrated in greatest abundance, and they stayed on till the 70th day. As early as 7 days after reinjection thickening and mild proliferation of the arachnoid were noted together with pial adhesion to, and cell infiltration in the optic nerve. In cases long followed up, severe pathologic changes of the chiasmal arachnoid were found to have acted injuriously upon the optic nerve. Clear demyelination of the optic nerve was noted in two cases respectively 80 and 90 days later. Damage of the optic nerve was most severe at the chiasm, but little seen in its intracranial and intraorbital portion. The retina always remained free from any involvement.

Vitamin A Deficiency Diet Group

Involvement of the arachnoid was observed in those cases killed between the 7th and the 21st days after first injection. Especially those killed on the 21st day showed somewhat more marked lesions than the normally-fed. Inflammatory cell infiltration in the arachnoid was not so marked in itself, but more prominent than in cases of the normally-fed. That is, endothelial cells were more swollen, and showed a tendency to rounding and dissociation. Also swelling of connective tissue fibers was more marked. According to Oelrich, lack of vitamin A in the feed produces higher susceptibilities to infection in rate and other animals than lack of other vitamins, and it is commonly known that epithelial tissue loses in resistance in the case of vitamin A deficiency. It is considered that lack of vitamin A makes the arachnoid as well more liable to be affected by inflammation.

After reinjection, by far more intense inflammatory reactions were noted in the arachnoid than after first injection. Swelling, proliferation and rounding of endothelial cells were observed together with swelling, degeneration and atrophy of connective tissue fibers. As to cell reaction, eosiniphils, plasma cells, mononuclears, fibroblasts and giant cells appeared in greater abundance. Vessel walls, too, were more severely damaged. Though Zilva, Werkman, Simola and Ito stated something to the following effect that there was no special relationship between vitamin A deficiency and immunity reaction, by far more advanced arachnoid damage was thus seen in the vitamin A-lacking animals than in the normally-fed in the present experiments. This severe arachnoid reaction after reinjection may be mainly attributable to the increased allergic action; it cannot be fully explained by decrease of tissue resistance due to lack of vitamin A alone.

As to degeneration of the optic nerve, Marchi's balls appeared in vitamin A-lacking animals earlier than in the normally-fed after first injection, and after reinjection they were demonstrated in much larger numbers in the former than in the latter. And so vitamin A deficiency seems also to hasten degeneration of the optic nerve. These results of the present experiments are in accord with a report of Mellanby, who experimentally proved that vitamin A was needed in the metabolism of the brain.

Vitamin B_1 Deficiency Diet Group

Exudative cells hardly appeared in the immediate period after first injection, and even two or three weeks later only pseudoeosinophils and small round mononuclears were noted in small numbers. Thickening of endothelial cell layers, and swelling of connective tissue fibers were as mild as in cases of the normally-fed.

Seven days after reinjection enlargement and dissociation of arachnoid endothelia were noted together with swelling of connective tissue fibers. These connective tissue fibers also showed partial adhesion to the pia mater. Prominent perivascular infiltration, too, was noted. Exudative cells consisted of eosinophils and small round mononuclears, and cell infiltration was conspicuous in the pia mater, optic nerve and interstitial tissue. Thus, inflammatory cell and tissue reactions were strong in the present experiments, though Katsuro stated after experimenting with the salmonella typhosa that vitamin B_1 deficiency lowered the produotion of agglutinins in rabbits.

After reinjection marked perivascular cell infiltration, congestion of small blood vessels, subarachnoid effusion, and cell infiltration in the pia mater and interstitial tissue of the optic nerve were observed, but it was obvious that these inflammatory changes had spread from the primary lesions of the arachnoid to the optic nerve. As a whole, however, tissue reactions of the arachnoid in the case of vitamin B_1 deficiency was not so remarkable as in that of vitamin A deficiency, but surely of higher degree than in cases of the normally-fed.

The appearance of Marchi's balls, hence degeneration of the optic nerve was most conspicuous in this group. This fact indicates the indispensability of vitamin B_1 in

the metabolism and functioning of the optic nerve; the myelin sheath of the optic nerve seems to be rendered very susceptible to damage by the lack of this vitamin.

What part of the optic nerve most readily falls into degeneration? In one case in the present experiment only the fiber bundles along the central axis were affected by Marchi degeneration. This degeneration extended from the optic tract through the chiasm to just prior to the optic disk, and all along the way strictly limited to the central axis. This finding, though obtained in one case only, may suggest the presence in the rabbit of the papillomacular bundle which is less resistant than the peripheral fibers. It seems that this less resistant bundle was further weakened by lack of vitamin B_1, and finally selectively damaged by adhesion, oppression, and disturbance of the intracranial fluid circulation. It is of course too hasty to conclude from this case alone that the central axial or any other specific fibers suffer more easily from lack of vitamin B_1, but the clinical picture of this case was very similar to that of axial optic neuritis of man.

The severest damage was seen at the optic chiasm, but it is quite conceivable that the chiasmal arachnoid is easily damaged by vaccines or any other toxins contained in the stagnant intracranial fluid in the adherent subarachnoid space, for the optic chiasm is the place where the intracranial fluid is usually present in greatest abundance. The fact that the anterior part of the optic chiasm is most susceptible has been borne out by other experiments with toxins conducted in our laboratory.

Discussion

Systemic and Optic Nerve Disturbances as Side Reaction of Antirabic Vaccination

The well-known side reactions of antirabic vaccination are quadriplegia and optic nerve damage, and also inflammatory demyelinating lesions are demonstrable in autopsied cases. Though these demyelinating lesions do not show a special preference for locality, they usually develop in the vicinity of cerebral ventricles. It may therefore be supposed that the intracranial fluid plays some important role in demyelination. Moreover, these lesions are not limited to the brain alone; they occasionally spread to the spinal cord through the subarachnoid space, and attack the gray matter near the central canal.

Retrobulbar neuritis produced as a result of antirabic vaccination is accompanied by arachnoid lesions, just as when caused by other etiologic factors, and clinically operative removal of the thickened and proliferative arachnoid results in improvement of eyesight. Also in the majority of clinical cases treated in our clinic arachnoid lesions have been demonstrated, and so the existence of arachnoiditis in postvaccinatory retrobulbar neuritis may be assumed as quite possible.

Shiina who had studied postvaccinatory quadriplegia stated that meningitis preceded perivascular round cell infiltration, and intense inflammation led to demyelination.

Recently we have had an opportunity to witness quadriplegia, impairment of

eyesight and the chocked disk in a dog subjected to antirabic vaccination. On autopsy advanced arachnoiditis was demonstrated together with lesions in the brain, spinal cord, and optic nerve.

In the present investigation the vaccine was introduced through an unusual route, but the results obtained indicate that arachnoiditis is an important factor in the development of lesions in the optic nerve.

Relation between Arachnoiditis and Retrobulbar Neuritis

Reactions produced by a single injection of antirabic vaccine were limited to slight thickening of the arachnoid, and mild cell infiltration therein and moreover, these finding were obtained only in the immediate period after injection. As early as 4 weeks later the cell reaction had already vanished, and neither swelling nor proliferation of the arachnoid was observable. The optic nerve, too, was now free from Marchi's balls. It may be considered that though the vaccine, borne by the intracranial fluid circulation, exerted a direct action upon the arachnoid and optic nerve, this action was not strong enough to elicit an intense reaction. The arachnoid was so mildly inflamed that the intracranial fluid circulation remained almost unaffected. A small number of Marchi's balls were noted in the optic nerve during a certain period after injection, it is true, but after all, antirabic vaccine, when injected only once, was incapable of causing advanced degeneration of the optic nerve. With this antirabic vaccine primary inflammatory reaction of the arachnoid was more noticeable than involvement of the optic nerve. By the way, fact that Marchi's balls appeared in very small numbers only at the optic chiasm 4 weeks after injection shows that hematogenic spread of the vaccine to the optic nerve is rather negligible.

Compared with first injection reinjection of the vaccine called forth extremely intense reactions from the arachnoid and optic nerve. Swelling, enlargement, and inflammatory cell reaction of the arachnoid were clearly due to allergy. The cell reaction was at its height from the 7th day to the 3rd week after reinjection, and after then exudative changes passed into the proliferative. Nine weeks later proliferation, thickening and adhesion of the arachnoid were clearly observable.

Marchi's balls appeared in the optic nerve soon after reinjection. This degeneration of the optic nerve was not entirely attributable to involvement of the myelin sheath in antigen-antibody reaction; allergic reactions in the arachnoid adjacent to the optic nerve, and in the interstitial tissue of the same nerve seem to have also participated in bringing it about.

These severe pathologic changes observed after reinjection were surely caused by antigen-antibody reaction, but this reaction does not account for the fact that Marchi's balls were seen in the optic nerve continuously till as late as the 7th week after reinjection; this must be explained by disturbed metabolism and malnutrition of the optic nerve, which resulted from arachnoid adhesion after allergic inflammation, disturbance of the intracranial fluid circulation, direct oppression by the proliferating

tissue of the optic nerve, and obstruction by the same of the blood circulation. These above-mentioned disturbing factors were really responsible for continuous appearance of Marchi's balls, and demyelination in the optic nerve.

Destruction of the optic nerve myelin sheath is thus caused not only by solitary involvement of the optic nerve, but also by thickening, proliferation and adhesion of the arachnoid adjacent to the nerve, and by disturbance of the intracranial fluid circulation. By the way, arachnoid changes were most conspicuous at the chiasm, just as in Iwasa's experiments, and pathologic findings of the chiasmal arachnoid were nearly the same as those obtained in clinical cases at the time of craniomy.

Ito and Nakajima once stated elsewhere that chiasmal arachnoiditis had little chance of involving the optic nerve, but in the present investigation a mutual parallel relationship was established between lesions of the arachnoid and optic nerve. Inflammatory reaction of the arachnoid was definitely seen spreading to the interstitial tissue of the optic nerve in the immediate period after reinjection, and so it cannot be that the arachnoid and optic nerve separately and independently gave their own responses to reinjection. Inflammation of the arachnoid seems to stand in an important relationship with degeneration of the optic nerve myelin sheath.

From what has been said a conclusion may be drawn that proliferation and adhesion of the chiasmal arachnoid continuously exert an injurious influence on the optic nerve, and finally damage its myelin sheath. It is far from the present author's intention, however, to assert that blocking and disturbance by arachnoid adhesion of the intracranial fluid circulation, and oppression by the same of the optic nerve are exclusive etiologic factors of optic nerve degeneration; he only wants to say that this mechanical factor should be taken as one of its causes.

At present in our laboratory experiments are being conduction on the effects on the optic nerve of arachnoiditis experimentally produced with talcum powder, along with physico-chemical investigations into the metabolism of the intracranial fluid and optic myelin sheath. These studies, when completed, will offer a more definite solution to this problem concerning the relation between arachnoiditis and retrobulbar neuritis.

The present investigation also proved that avitaminosis would increase susceptibility of the optic nerve, and contribute to intensification of its damage.

Conclusion

1. Antirabic vaccine, when reinjected into the cisterna magna of the rabbit, produced by far more severe allergic arachnoiditis than when firstly injected. After reinjection inflammation of the arachnoid instantly spread to the optic nerve. The arachnoid showed thickening and proliferation, and its adhesion to the optic nerve disturbed circulation of the intracranial fluid, and finally produced persisting degeneration of the optic nerve. Marked demyelination was pathohistologically demonstrated.

2. Allergic arachnoiditis caused by reinjection of the vaccine was more severe in

rabbits fed on vitamin A deficiency diet than in the normally-fed. Degeneration of the optic nerve was clearly aggravated by lack of vitamin A, but not so much as by lack of vitamin B_1.

3. Allergic arachnoiditis observed in rabbits fed on vitamin B_1 deficiency diet was not so severe as that in those lacking in vitamin A, but certainly more intense than in the normally-fed. In these animals thickening, proliferation and adhesion of the arachnoid were noted, and degeneration of the optic nerve was most striking.

4. The present investigation has shown that disturbance of the intracranial fluid circulation must be reckoned as one of the etiologic factors of retrobulbar neuritis due to meningitic neuritis.

5. The present investigation has also shown that deficiencies of vitamin A and B_1 are important factors responsible for involvement of the arachnoid and optic nerve.

(Published in Acta Ophth. Jap. 63:746, 1959 in Japanese.)

Photographic Illustrations (I). Normally-fed Rabbits

Photo. 1. Exudative cells in the arachnoid 24 hours after first injection. 10×10

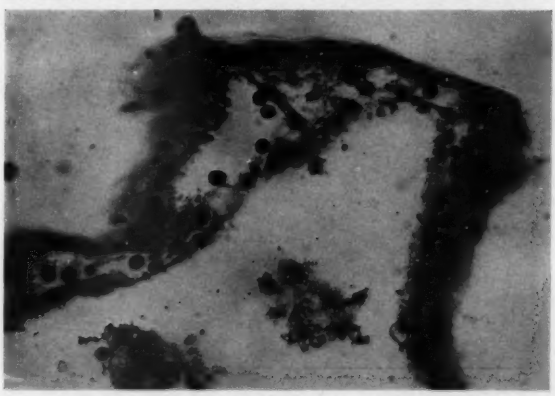


Photo. 2. Eosinophils in the arachnoid 24 hours after reinjection. 10×40

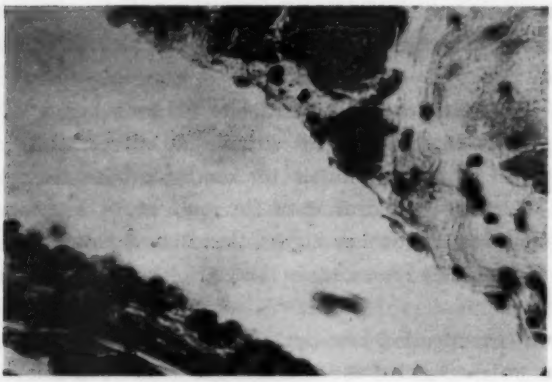


Photo. 3. Giant cells in the chiasmal arachnoid 24 hours after reinjection. 10×40

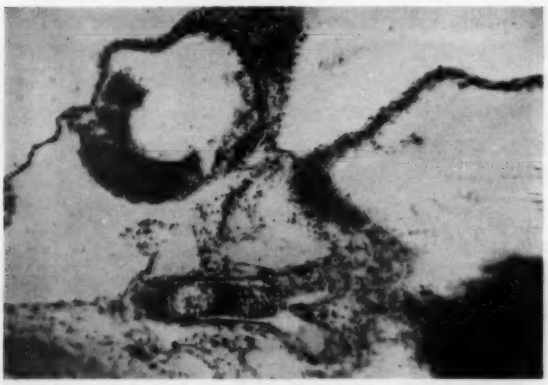


Photo. 4. Perivascular cell infiltration and hemorrhages in the arachnoid adjacent to the cisterna chiasmatica 14 days after reinjection. 10×10

Photo. 5. Arachnoid thickening, proliferation and adhesion at the optic chiasm 2 months after reinjection. 10×10

Photo. 6. Marchi's balls at the optic chiasm 14 days after reinjection. 10×10

Photo. 7. Marchi's balls in anterior part of the optic chiasm 21 days after reinjection. 10×10

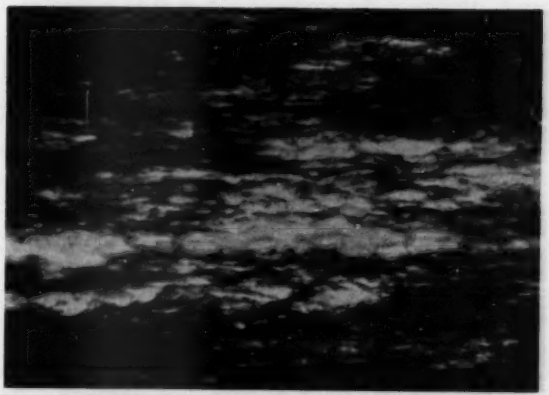
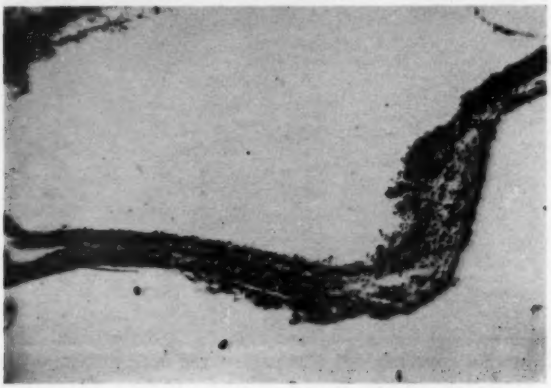


Photo. 8. Demyelinating lesions in anterior part of the optic chiasm 80 days after reinjection. 10×10

Photo. 9. Demyelinating lesions in anterior part of the optic chiasm 90 days after reinjection. 10×40

Photographic Illustrations (II). Rabbits Deficient in Vitamin A



Photr. 1. Picture of the arachnoid 7 days after reinjection. 10×10

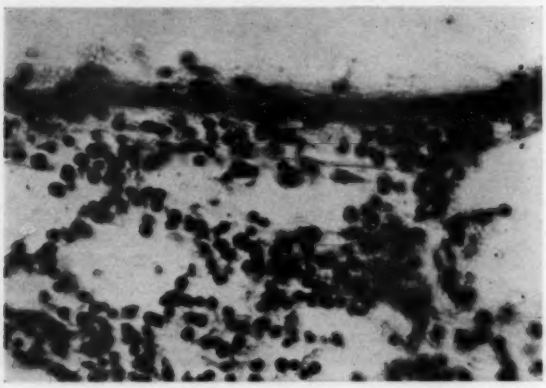


Photo. 2. Picture of the arachnoid 7 days after reinjection. 10×40

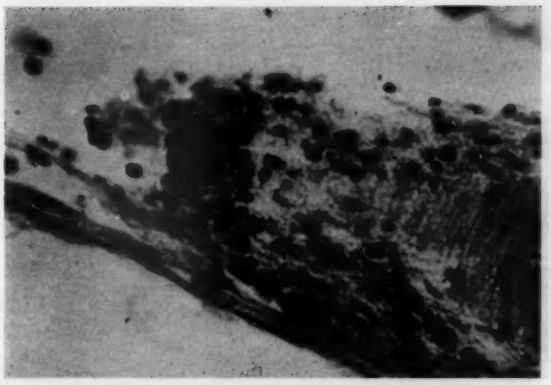


Photo. 3. Giant cells in the arachnoid 14 days after reinjection. 10×40

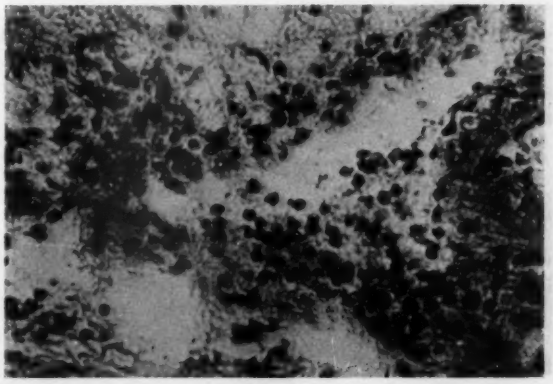


Photo. 4. Swelling of the arachnoid and connective tissue fibers, and perivascular cell infiltration. 10×40

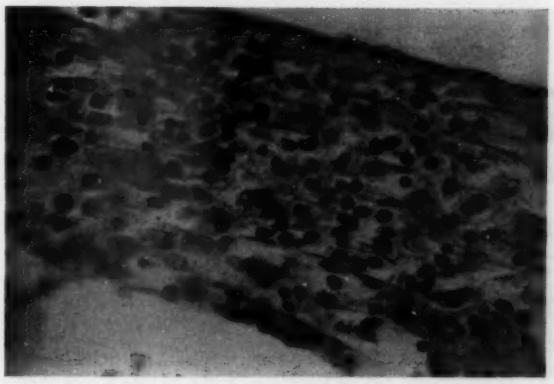


Photo. 5. Arachnoid thickening and proliferation 21 days after reinjection. 10×40

Rabbits Deficient in Vitamin B_1

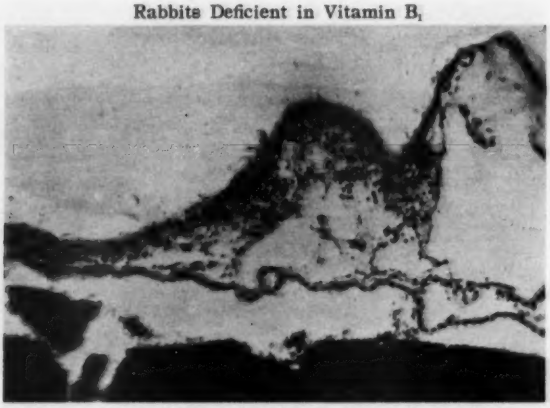


Photo. 6. Exudative cells in the arachnoid, and subarachnoid hemorrhages 7 days after reinjection. 10×10

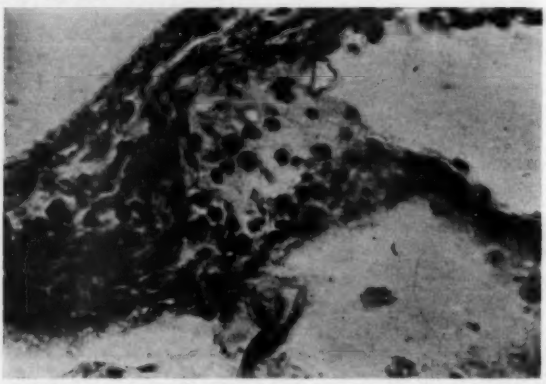


Photo. 7. Infiltrating eosinophils and lymphocytes in the arachnoid 7 days after reinjection. 10×40

Photo. 8. Perivascular cell infiltration in the chiasmal arachnoid 7 days after reinjection. 10×10

Photo. 9. Arachnoid thickening and adhesion to the optic nerve, and exudative cells 14 days after reinjection. 10×10

Photo. 10. Cell infiltration in the arachnoid, and adhesion to the optic nerve 14 days after reinjection. 10×10

Photo. 11. Arachnoid thickening and proliferation 21 days after reinjection. 10×40

Rabbits Deficient in Vitamin A

Photo. 12. Marchi's balls at the optic chiasm 7 day after reinjection. 10×10

Photo. 13. Marchi's balls in anterior part of the optic chiasm 14 days after reinjection. 10×10

Rabbits Deficient in Vitamin B_1

Photo. 14. Marchi's balls in anterior part of the optic chiasm 7 days after reinjection. 10×10

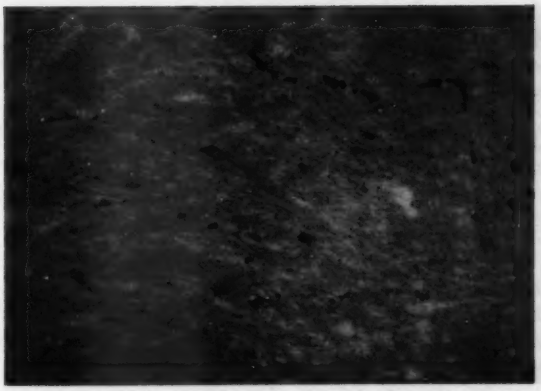


Photo. 15. Marchi's balls at the optic chiasm 7 days after reinjection. 10×10

Photo. 16. Marchi's balls in the optic nerve 14 days after reinjection. Note that central axial fibers alone are involved. 10×10

Photo. 17. Marchi's balls in the optic nerve 14 days after reinjection. Note that central axial fibers alone are involved. 40×10

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THE RETINAL BLOOD PRESSURE IN CHOKED DISC

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Choked disc is produced in many conditions. The retinal blood pressure in choked disc due to reducing process of the intracranial space by brain tumor was measured. There are many reports about it. However, there are still some disagreements and unsolved problems on the results of measurements and the interpretation.

Method

The blood pressure in the central retinal vessels. After measuring the intraocular tention with Schiötz's original tonometer, the retinal venous blood pressure (RVP), then the diastolic blood pressure in the central retinal artery (RAP min) and the systolic (RAP max) were measured with Mikuni's ophthalmodynamometer in the spin position. The intraocular pressure seeked from the standard intraocular pressure line corresponding the nagative pressure, which was produced by the ophthalmodynamometer, indicates the blood pressure of the central retinal vessels.

Stages of choked disc. The patients with choked disc were divided into 3 stages according to Hippel's classification.

First stage (I) is an early choked disc. The optic disc is slightly swollen and reddish. However, the disc and adjacent retina are transparent and the lamina cribrosa is well visible.

Second stage (II) is a fully developed choked disc. The swelling of the disc in-

creases and the physiologic cup becomes filled in with gray tissue. The margins become blurred and the lamina cribrosaunvisible. Hemorrhages and neovascularization may occur.

Third stage (III) is an atrophic or late choked disc. The swelling of the disc subsides and optic atrophy developes.

When choked disc is found only in one eye, O stage is named.

Results

The cases were 51 patients (102 eyes), 32 of male (64 eyes) and 19 of female (38 eyes), with choked disc who the retinal blood pressure was measured and the craniotomy was performed at Niigata University Hospital from 1952 to 1957.

As to the systemic blood pressure, the diastolic under 140 mmHg was measured in 47 cases (92.1%) and the systolic in 47 (92.1%).

The intraocular pressure averaged 14.28±0.15 mmHg.

The results of measurements are shown in Table 1.

Table 1.

Stage		Cases (Eyes)	RAP mmHg		Ratio of RAP to BP %		RVP mmHg	RAP (Min) —OT mmHg	RAP (Min) RVP
			Max.	Min.	Max.	Min.			
Normal		73	135.0—106.0	89.0 —55.0	73.4 —52.0	67.1 —50.0	94.0 —58.0	35.5 —11.0	2.84—1.48
		(146)	119.3± 1.78	72.3 ±1.77	62.31±3.56	55.70±1.56	74.47±1.77	25.08±1.38	2.24±0.0791
Choked Disc	0	4	90.0 —67.0	50.0 —43.0	70.4 —55.8	62.5 —57.0	23.0 —22.0	35.0 —27.5	2.17—1.95
		(7)	81.25±16.5	46.25±4.69	65.15±10.4	59.9 ±5.71	22.66±2.18	31.87±5.45	2.02±0.57
	I	14	92.5 —64.5	58.0 —36.0	78.2 —27.5	73.0 —51.5	30.0 —16.0	43.0 —24.0	2.25—1.50
		(18)	80.41± 4.03	47.9 ±2.35	66.9 ±66.9	59.6 ±3.09	25.55±3.16	33.61±4.66	1.87±0.14
	II	25	99.0 —57.5	57.5 —27.0	78.2 —55.0	70.6 —42.8	31.0 —15.0	46.5 —12.0	2.00—1.50
		(40)	77.76± 3.68	43.43±2.71	65.29±1.88	57.58±2.14	24.02±2.26	29.22±2.67	1.77±0.11
	III	23	90.0 —54.0	50.0 —30.0	65.5 —50.0	62.5 —42.9	26.0 —14.5	35.0 —17.0	2.53—1.57
		(40)	67.19±2.83	38.66—1.94	59.43±13.3	51.83±1.47	18.2 ±0.88	24.81±1.79	2.06±0.09

The blood pressure in the central retinal arterv. With progress of stage, RAP (max) (min) tend to be decreased. Statistically being no significant difference, the mean values of RAP were elevated in O and I stage, then decreased in II and lowest in III.

The blood pressure in the central retinal vein. RVP was possible to be measured in 30 cases (60 eyes) of 51 (102). Spontaneous venous pulsation was observed in 8 cases (16 eyes). The other 13 cases (25 eyes) were impossible to measure RVP owing to retinal hemorrhages or maked edem. Statistically being no significant difference, RVP was raised in O stage, moreover I or II and lowest in III.

The ratio of the retinal arterial to brachial blood pressure. In both the systolic and diastolic blood pressure, it was high in O and I stage, decreased in II and markedly low in III.

Difference between the retinal diastolic blood pressure and the intraocular pressure. It was high in O and I stage, decreased in II and least in III. The intraocular pressure was not changed in stages. Therefore the changes of the differences were due to changes of RAP (min).

The ratio of the retinal arterial diastolic to the retinal venous diastolic pressure. No significant change was observed in stages.

Comparison in cases with different stage in each eye. Many patients have choked disc of the same stage in both the eyes. There are not few cases with choked discs of different stages.

The averages of each stage in these cases are shown in Table 2.

Table 2.

Case	Stage	Eyes	RAP mmHg		Ratio of RAP to BP %		RVP	RAP (Min) —OT	RAP (Min) / RVP
			Max.	Min.	Max.	Min.	mmHg	mmHg	
1~ 4.	0	4	81.25	46.25	65.15	57.10	22.66	31.87	2.00
	I	4	83.37	46.75	66.80	57.70	25.00	32.37	1.89
5~ 8.	I	8	81.75	49.31	65.81	59.32	26.0	35.06	1.96
9~13.	I	5	75.6	46.3	68.28	61.94	24.5	32.3	1.88
	II	5	71.8	44.2	65.48	58.94	23.3	30.0	1.83
14~28.	II	30	79.20	43.28	64.93	56.87	24.95	29.13	1.75
29~33.	II	5	75.1	43.6	66.08	60.48	28.0	29.0	1.83
	III	5	67.1	39.8	60.22	53.70	18.5	25.4	2.09
29~33.	III	34	68.95	39.8	59.58	52.51	18.37	25.4	2.06
51.	I	1	86.0	50.0	70.5	58.1	30.0	33.5	1.66
	III	1	80.0	46.0	65.5	53.5	22.0	29.5	2.09

RAP (max) (min) were high in O and I stage and decreased in II and III stage. The same tendency was observed in the ratio of RAP to the brachial blood pressure. RVP was high in I and II stage and decreased in III. The difference between RAP (min) and the intraocular tention was high in O and I stage, then in II and lowest in III. The ratio of RAP (min) to RVP was almost same in each stage.

Comparison before and after the operation. In 13 cases the retinal blood pressure was measured before and after the operation of brain tumor. After the operation both RAP and RVP were decreased to the normal value in some cases and under the normal in the others, especially being remarkable in the cases with marked optic atrophy.

In the cases with choked disc of I stage before the operation the optic discs became mostly to the normal, rarely to II and III stage after the operation.

In the cases with choked disc of O stage the optic discs became to I stage and and returned to O stage or to II stage.

In the cases with choked disc of II stage the optic discs became to I stage and then the normal in some cases and mostly to III stage.

In the cases with choked disc of III stage the optic discs were still in III stage.

Relation on the focal side. In 15 cases with brain tumor in the right side of the brain RAP was higher in the right eye than the left in 8 cases, lower in 3 and same in 4.

In 13 cases with brain tumor in the left side of the brain RAP was higher in the left eye in 7 cases, lower in 2 and same in 4.

Comment

Relation on the retinal arterial blood pressure and stages of choked disc

There are many reports about RAP in choked disc due to increased intracranial pressure.

Deyl (1912) stated that RAP in 3 cases with choked disc was elevated probably due to arteriosclerosis and RVP might be decreased. Bailliart (1922, 1923) described that the increase of RAP was observed in many cases. Rasvan (1926) wrote that RAP (min) was almost normal and RAP (max) was slightly raised. Berens et al. (1928) mentioned that an important diagnostic point was the disproportionate increase in the retinal diastolic pressure over the brachial diastolic pressure, for when edema was fully developed the retinal pressure was likely to fall.

Then Bailliart (1929, 1930) reported that elevated RAP returned to the normal when choked disc was developed. Damel (1930), Tille (1934), Guillst and Paillas (1935) agreed with the finding. Claude, Lomache and Dubar (1928) thought the decrease was due to arterial insufficiency. Bailliart (1929, 1953) also agreed with the explanation.

Sobanski (1934) presented an new theory that RVP was always elevated and RAP not always raised in choked disc due to increased intracranial pressure and a rise of RVD might be a main condition leading to formation of choked disc. Lauber (1937) added that low RAP was a main factor to cause choked disc. However, Kyrieleis (1937) and Marchesani (1938) objected to this theory.

Streiff and M. Monnier (1946) wrote a paper that the brachial blood pressure was normal in 20 cases of 30 with choked disc due to intracranial neoplasms, decreased in 2 and moderately increased in 7, while RAP was discordantly decreased in 9 and discordantly increased in 5.

As above mentioned, there are disagreements about the height and the interpretation of RAP in choked disc. However, there is no detail study on RAP in choked disc.

In our results, RAP (max) (min) were already elevated before the onset of choked disc, still higher in the early stage, then decreased to the normal in the eflorescent

stage and below the normal in the late stage. Interpreting the results in the literature from our experiment, RAP appeared to be high in the reports measured mainly in the early stage and low in the eflorescent or the late stage. It was made clear that RAP was depend on stages of choked disc.

It is quite easy to diagnose choked disc in the eflorescent stage but requires a great deal of skill and sometimes an obsevation by means of a slit lamp in the early stage. Our results that RAP was elevated already before the onset of choked disc and ratio of RAP to the brachial blood pressure also high corresponds to the Berens's opinion that the disproportionate increase in RAP (min) over the brachial diastolic blood pressure was important to establish an early diagnosis, for when edema was fully developed the retinal pressure was likely to fall.

The results that RAP decreased to the normal in the eflorescent stage corresponds to Bailliart and Daniel's reports that elevated RAP again returued to the normal when choked disc was developed.

The retinal venous blood pressure

The spontaneous venous pulsation is often observed in the normal eyes and it reveals that RVP (min) is lower than the intraocular pressure. Lauber and Sobanoski mentioned that RVP became to be raised with elevation of the cerebrospinal pressure and the spontaneous venous pulsation did not occur. Sobanski proved the fact that the ratio of RAP (min) to RVP (min) was from 1.9 to 3.0 in the normal persons and under 1.5 RVP was relatively increased, resulting in development of choked dise. Lauber also agreed with it.

However, Levina (1939) described that RVP was elavated in the early stage of choked disc and decreased in fully developed stage and the decrease was not a cause of choked disc but the result.

In our cases the ratio of RAP to RVP was lower than in the normal persons. So RVP was always comparatively higher than RAP in each stage of choked disc. It was also proved by the fact that the spontaneous venous pulsation was found only in 15.6% of the patients with choked disc, being in 48.4% (Yoneyama) and 35.4% (Nakashizuka) of the normal persons.

Therefore it is an important findnig in the early diagnosis of choked disc that RVP was elevated not only in the early stage but also already before the onset.

Relation on the locality of brain tumor

There are different opinions about the diagnostic value of the retinal blood pressure for the locality of brain tumor.

Coppez (1926), Rasvan (1926), Bailliart (1929), Magitot (1926), Pereyra (1931) and Spinelli (1933) found that the increase of RAP in choked disc was observed in both the eyes, while Gallois (1929), Bauwens (1928), Winther (1930), and Asher (1937) recognized it only in the side of brain tumor. However, Asher found it in the other side in 2 cases of cerebellar tumor.

De Morsier, Monier and Streiff (1938, 1939) described that RAP was normal or low in the brain tumors located in the anterior and middle cranial fossa and elevated in the tumors of the cerebellum and posterior hemisphere located in the posterior.

In our results there were more cases with higher RAP in the side of the tumor. However the difference was quite few and there were also higher RAP in the other side and same in some cases. Therefore the ophthalmodynamometry seems to be almost useless to determine the locality of brain tumor.

Relation on the operation of brain tumor

We know the cases that the brain tumor is well removed but the visual acuity is going to be lost even after the operation. So the brain tumor should be operated as soon as possible and the ophthalmodynamometry is important for the early diagnosis.

As choked disc in tumor is due to increased intracranial pressure, the removal of the brain tumor leads to the decrease of the intracranial pressure and then the recovery of the choked disc.

Therefore the elevated RAP was decreased with the recovery of choked disc by the operation.

Mechanism of choked disc

There are a number of assumptions on the mechanism of choked disc.

Sobanski found that the ratio of RAP was 1 1.7-2.6 in the normal persons and the alteration of the proportion beyond 1 : 1.1-1.5, namely the relative increase of RVP, leaded to choked disc. Lauber added that low arterial pressure was of importance not only for formation but also for the further development of choked disc.

Kyrieleis disagreed with Sobanski's thought that choked disc was not due to the alteration of the proportion of RAP to RVP but changes of the optic nerve. Marchesani (1938) also stated that the alteration of the proportion of RVP to RVP was not the cause of choked disc but the result.

In our cases the ratio of RAP (min) to RVP (min) was not particularly changed in each stage of choked disc, compared with the healthy subjects. RAP was nearly parallel with RVP and the apperance of the optic disc.

As Marchesani mentioned, the alteration of RAP might be not the cause of choked disc but the result.

Streiff et al. wrote that the alteration of RAP was not directly depend on the reducing process of the intracranial space but indirectly on the central regulation of the cerebral circulation. If so, some changes of RAP are main and must be local with changes of the optic nerve in choked disc.

Signification in differential diagnosis

As Streiff emphasized, the ophthalmodynamometer is important for the differential diagnosis between choked disc due to the increased intracranial pressure and papilledema due to systemic hypertention.

RAP in choked disc only slightly changed in most cases, being considerably low or

high in the exceptional cases. The brachial blood pressure was not changed. RAP in papilledema due to hypertention was always markedly elevated.

The measurment of the retinal blood pressure in 4 patients with essential malignant hypertention, who were hardly differentiated from choked disc due to brain tumor, were shown in Table 3.

Table 3.

Cases	BP mmHg		RAP mmHg		Ratio of RAP to BP %		RVP	RAP(Min) —OT	RAP (Min)
(Eyes)	Max.	Min.	Max.	Min.	Max.	Min.	mmHg	mmHg	RVP
4	220~192	140~128	161.0~137.0	106.0~93.0	74.4~72.0	74.7~70.2	26.0~2200	90.0~79.0	4.60~3.62
(8)	213.0	131.5	154.00	98.00	73.00	73.00	24.50	82.75	4.25

Compared with Table 2, RAP (max) (min) were almost twice higher in malignant hypertention than brain tumor. The difference between RAP (min) and the intraocular pressure also was about twice higher in the former than the latter. The ratio of RAP to the brachial blood pressure was remarkably higher in malignant hypertetion. Although RVP was elevated almost in the same degree in both malignant hypertention and brain tumor, the ratio of RAP to RVP was remakarbly higher in the former than the latter, showing marked elevation of RAP in the former.

Besides this finding, the systemic blood pressure and disturbances of the renal function are helpful for the differential diagnosis.

Since RAP is markedly higher in papillledema due to malignant hypertention than choked disc due to brain tumor by means of measurement of the retinal blood pressure, it is easy to differentiate them.

Summary

In 51 patients with choked disc caused by brain tumor, the retinal blood pressure was measured by Mikuni's ophthalmodynamometer. the results were as follows:

1. The retinal arterial blood pressure was already elevated before the onset of choked disc, still high in the early stage, then decreased to the normal in the eflorescent and below the normal in the late stage. The same was found in the ratio of the retinal to the systemic blood pressure in each stage of choked disc.

2. The retinal blood pressure was also elevated before the onset, moreover in the early stage, then decreased in the eflorescent stage to the level, which was a little higher than the normal, and below the normal in the late stage.

3. It is practically important for the early diagnosis of choked disc that the retinal blood pressure was elevated not only in the early stage but also already before the onset.

4. The earlier the craniotomy was performed in patients with choked disc, the better the prognosis for the visual acuity, Therefore the ophthalmodynamometry is

greatly valuable for the early diagnosis of the brain tumor.

5. The differential diagnosis of papilledema in malignant hypertention from choked disc in brain tumor was performed very easy by the ophthalmodynamometry, the retinal arterial blood pressure being markedly higher in the former.

6. The ophthalmodynamometry is useless to determine the locality of the brain tumor.

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A STUDY ON ACID MUCOPOLYSACCHARIDE OF OX AND RABBIT CORNEA

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Fractionation of bovine cornea based on isolation of acid mucopolysaccharides has yield three components, chondroitin sulfate, chondroitin and keratosulfate[1].

In previous papers it was reported by author and also by B. Wortman et al. that two components were detected on paper electrophoresis in the preparations of polysaccharides obtained from animal corneas, however, were poorly separated. It was impossible to obtain the satisfactory identification of these components from their electrophoretic mobility.

In present investigation polysaccharides are prepared by paper chromatography

and separation of three acid mucopolysaccharides is reported.

Methods

Isolation of mucopolysaccharides

1) Mucopolysaccharides of ox cornea were prepared by K. Meyer's method and fractionetad with ethanol. The first three fractions were precipitated at 20,30 and 40 per cent ethanol (Fraction 1, 2 and 3). The forth fraction, which was precipitated at 50 per cent ethanol was redissolved in pH 4.6 acetate-acetic acid buffer solution and re-fractionated at 40 and then 50 per cent ethanol (Fraction 4).

2) The slices of rabbit cornea were shaken with S^{35}-sulfate in the Krebs-Ringer bicarbonate solution at 37°C for 2 hours, rinsed with water and stored in aceton.

The dried sections were suspended in destilled water and ground by a glass homogeniser. Mucopolysaccharides were isolated from this liquid through the steps outlined by K. Meyer and fractioned with ethanol at 40 and 50 per cent concentration.

3) The dried rabbit corners were soaked in water and cut into small pieces. The sections were suspended in destilled water and ground by a glass homogeniser. To above solution an equal volum of 4 per cent NaOH solution was added and mucopolysaccharides were extracted in the refrigerator.

The viscous liquid was filtrated through glassfilter and the filtrate was neutralized with acetic acid.

The solution was dialysed against destilled water. The solids appeared during the dialysis were removed by filtration and this filtrate was concentrated by evaporation.

Mucopolysaccharides were precipitated with 2 volumes of ethanol in the presence of sodium chloride.

The precipitate was collected by centrifugation after 24 hours then washed with 80 per cent ethanol.

It was dissolved in a amount of water and the pH of the solution was brought to about 8.0 with NaOH solution.

After the undissolved matters were removed by centrifugation, trypsin was added. The mixture was coverd with toluen and incubated at 37.°C for 36 hours.

The solids were removed by filtration and an equal volume of pH 4.6 mixture of 5 per cent calcium acetate and 0.5 N acetic acid was added.

Polysaccharides were precipitated with 1.5 volumes of ethanol and collected after 24 hours by centrifugation.

The precipitate was washed with 80 per cent ethanol, and dissolved in a mixture of 5 per cent sodium acetate and 0.5 N acetic acid.

The solution was shaken with chloroform-amyl alcohol mixture and to the clear supernatant 1.5 volumes of ethanol were added.

The resulting precipitate was centrifuged, washed with 80 per cent ethanol and then redissolved in distilled water.

The solution was cleared by centrifugation at 16000 r.p.m and an equal volume of mixture of 10 per cent calcium acetate and IN acetic acid was added.

The mucopolysaccharides were precipitated with an equal volume of ethanol, collected after 24 hours, then washed with ethanol and dried.

4) The mucopolysaccharides of rabbit cornea were prepared by method of T. Sato.

The sections of corneas were suspended in water and grounded by a glas homogeniser. To above solution NaOH was added until the concentraion of solution was attained 0.5 per cent and then polysaccharides were extracted by heating at about 50°C for 15 minutes. It was deproteiniged by heat coagulation at 70°C and by saturation with ammonium sulfate. The solution was filtred and acid mucopolysaccharides were precipitated with toluidine blue from the filtrate.

The resulting precipitate was collected, washed with acid solution and dissolved in formamide.

The formamide-soluble fraction were precipitated with methanol in the presence of sodium chloride. Precipitate was dissolved in water and precipitations with methanol were repeated.

Acid mucopolysaccharides were obtained as calcium salts.

Paper chromatography

The solvent system employed was: n-propyl alcohol-M/15 phosphate buffer (35 : 65 or 30 : 70, pH 6.4) in ascending elution at 4°C (Kerby's method); Whatman No. 1 paper was used throughout; mucopolysaccharides were detected by metachromatic staining with toluidine blue or autoradiographic procedure on X-ray film.

Results and Discussion

Acid mucopolysaccharides of ox cornea

The results of typical preparations obtained by paper chromatography are shown in Fig. 1 and Fig 2. It is seen that the preparations show at least three demonstrable components in polysaccharide of ox cornea.

Fraction 1,2 and 3:

From the view point of the Rf value two metachromatic components were detected on the paper in the preparations of these fractions.

Fig. 1. Chromatograms of acid mucopolysaccharides of ox cornea. A solvent consisting of n-propylalcohol-M/15 phosphate buffer, 35:65 was utilized. The polysaccharide spots were detected by metachromatic staining with toluidine blue.

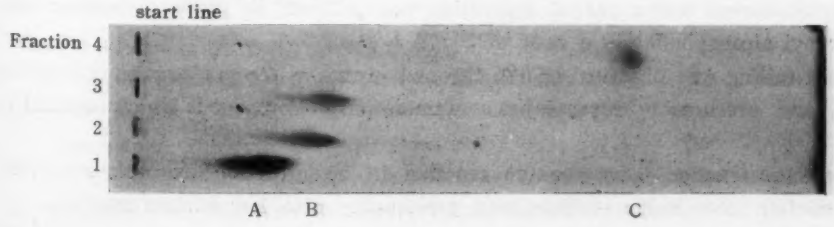


Fig. 2. Chromatograms of acid mucopolysaccharides of ox cornea.
Solvent: n-propylalcohol-M/15 phosphate buffer, 30:70.

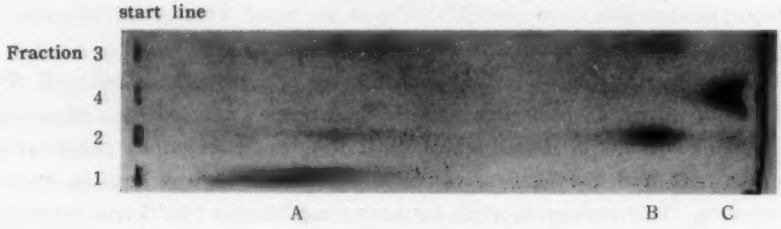


Fig. 3. Chromatogram of acid mucopolysaccharides of rabbit cornea.
Sections of cornea were incubated with S^{35}-sulfate and acid mucopolysaccharides were isolated by K. Meyer's method.
Solvent: n-propylalcohol-M/15 phosphate buffer, 35:65.
1: chromatogram (0-40 per cent fraction +50 per cent fraction)
2: its autoradiogram on X-ray film

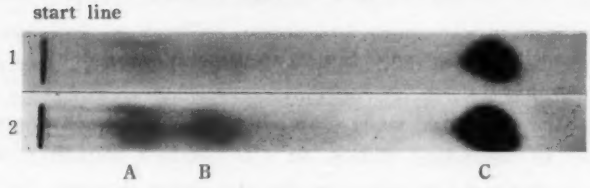


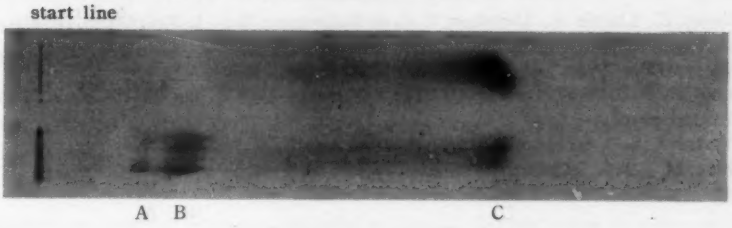
Fig. 4. Chromatograms of acid mucopolysaccharides of cornea.
1: Fraction 4 of ox cornea.
2: Fraction 3 of ox cornea.
3: Fraction I of ox cornea.
4: Acid mucopolysaccharides of rabbit cornea which prepared by method of T. Sato et al.

The separation of these two components proved extremely difficult by the use of paper electrophoresis and a partial separation was achieved by paper chromatography with n-propyl alcohol-buffer in a ratio of 30 : 70.

Marked tailing was observed on the chromatograms in the preparation of Fraction 2 and 3, and presumably represented contamination of component which detected in Fraction 1.

All of these fractions gave positive reaction in hexosamine, hexuronic acid tests, so that contain chondroitin sulfate and presumably also low sulfate fraction.

Fig. 5. Chromatogram of acid mucopolysaccharides of rabbit cornea.
Polysaccharides were extracted with dilute alkali and
isolated by method of preparation 3) as described above.
Solvent: n-propylalcohol-M/15 phosphate buffer, 35:65.
1: Fraction 4 of ox cornea.
2: Acid mucopolysacchariedof rabbit cornea.



Fraction 4:

This fraction gave positive reactions in hexosamine and sulfate tests and also showed strong Molisch-reaction. The content of hexuronic acid was very low. This fraction contains probably keratosulfate on the base of its composition, and from this fraction one metachromatic compound was detected on the paper.

Acid mucopolysaccharides in rabbit cornea

The pattern of polysaccharides isolated through the steps outlined by K. Meyer was shown in Fig. 3 and was similar to that of ox cornea.

Three metachromatic compounds were observed on the paper in the preparation of total mucopolysaccharides of rabbit cornea and radioactive sulfate was detected on all three of these compounds.

It was also seen that a metachromatic component which had the largest Rf value among these components failed by saturation with ammonium sulfate in the course of preparation by method of T. Sato et al. It is considered that this component may be filmly bound to protein and is appear to be keratosulfate (Fig. 4).

A results of preparation of polysaccharide extracted with dilute alkali is shown in Fig. 5. In previous paper it was reported by author that the extract with dilute alkali contained two migrating components on paper electrophresis which, however, very poorly separted. In present investigation the polysaccharides were prepared by paper chromatography and a satisfactory separation of three metachromatic components was obtained.

Summary

From ox and rabbit cornea, acid mucopolysaccharide fractions were isolated and prepared by Kerby's method on paper chromatography.

The results obtained were as follows:

1. Three metachromatic components were demonstrable by paper chromatography in polysaccharide fractions obtained from ox cornea by method of K. Meyer et al..

2. The pattern of acid mucopolysaccharide in rabbit cornea were similar to that in ox cornea from the view point of paper chromatographic findings.

(Published in Acta O. Jap. 63:543, 1959 in Japanese.)

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Editorial News

The Sixty Third Japan Ophthalmological Congress was held at the Ochanomizu Women's University in Tokyo, on March 29, 30 and 31,1959. The Chairman was Prof. Hogara Hagiwara M.D. of University of Tokyo. Besides ninety ordinary speaches one special speach and a symposium were presented.

Special speach :

On Behçet's Syndrome Prof. Hogara, Hagiwara, University of Tokyo

Diseases included in the so-called muco-cutaneo-ocular syndrome are divided into 3 groupes as follows :

Ⅰ) Erythema exsudativum multiforme syndrome, Erythema exsudativum (Hebra), Dermatostomatitis (Baader), Stevens-Johnson's disease, Ectodermose érosive pluriorificielle (Rendu)

Ⅱ) Behçet's syndrome and its inocomplete forms, Behçet's Syndrome, Franceschetti-Valerio's syndrome, Ulcus vulvae acutum (Lipschütz), Aphthosis acuta (Neumann), Aphthosis chronica recidiva (Kumer)

Ⅲ) Reiter's disease

Among the symptoms of Behçet's syndrome the eariest and the most frequent is oral aphtha, which is followed by skinsymptoms, and the ocular and genital affections in general appears in much later stage. Nearly all of the skin eruptions show themselves in the form of erythema nodosum. Ulcer of the female genitals never appears before the first menstruation and affer climacteric, and, what is more interesting, the symptom grows worse in the stage of ovulation and gets better at the beginning of menstruation.

The serum protein test reveals usually decrease of the A/G ratio, provided that the polysaccharides combined with protein being increased in the α_2 and β fractions of globulin.

Depression of the pancreas function and hypoflavinosis are confirmed.

CRP-test is positive in the complete type of Behçet's syndrome in 61.5% and in incomplete type in 33.3%.

As to the ocular symptoms the recurrent iritis presents itself not always under the form of hypopyon, but of suspension of leucocytes in the anterior chamber or in serous and fibrinous forms. Ocular appearances can be divided into the following 5 stages :

Ⅰ) General affections without ocular symptoms.

Ⅱ) Changes in the anterior segment of the eye-ball with a slight change in the fundus,

Ⅲ) Fundus changes become pronounced and changes of the choroid involve the retina.

Ⅳ) Fundus changes result in scar and marked destruction of the macula appears.

Ⅴ) Optic atrophy, detachment of the retina and secondary glaucoma produce blindness.

Histologically one preparations of erythema nodosum shows an image of acute inflammation and the other chronic productive. Tissue of the uvea is better preserved than the author expected. Exudates from the pars plana of the ciliary body formed a heavy membrane, which, in the course of shrinking, pulled the retina from the fundns up to the posterior surface of the crystalline lens. The retina in such an old stage has already suffered severe lysis and loss of celullar elements remaining its supporting tissues alone. Moreover, in such an old case there can be found an image of fibrinoid swelling and necrosis of the vascular walls, accompanying hemorrhages and edema, altogether suggesting antigen-antibody reaction, probably caused by the destroyed substance of the choroid and the retina.

Symposium: EKC

1. The etiology of EKC, with special reference to Adenovirus type 8
   Yukihiko Mitsui et al., Prof. of Tokushima Univ.
2. Studies on the relationship between Adenovirus and EKC, and on the superficial punctate keratitis
   Seiji Sugiura et al., Assistant Prof. of Univ. of Tokyo
3. Tissue cultural and electro microscopic studies of Adenovirus
   Syozo Oishi et al., Prof. of Yamaguchi Med. School

Ordinary Speaches

1. Quantum biophisics of vision
   Keisuke Abe, Nara Med. College
2. Study on objective measurement of visual acuity
   Ryoichi Yamaji et al., Osaka Med. College
3. Experimental study on visual acuity for a moving object
   Teruo Motohashi, Keio Univ.
4. Retinal representation in the lateral geniculate body
   Tadashi Fujino & Yonan Go, Nagasaki Univ.
5. New Quantitative test of colour vision
   Takuji Yamamoto, Yokohama Univ.
6. Colour vision survey on school children
   Akio Majima, Akira Nakajima, and Hiroshi Ichikawa, Nagoya Univ.
7. Development of the depth perception for near and distant object in the childhood
   Jisuke Kitano, Kyoto Pref. Med. Univ.
8. The false projection
   Ryohei Umeno, Kyushu Univ.
9. Studies on amblyopia
   Yoshimasa Watanabe, Okayama Univ.
10. Statistic investigation of the partially sighted
    Kazuo Hamano & Noburo Oyama, Nippon Univ.
11. Studies on night myopia
    Hiroshi Murata, Fukushima Med. College
12. Analytical Studies on refractive elements of the human eye by phacometry
    Hiroshi Tani, Tokyo Med. and Dent. Univ.
13. Histological study of myopic eye
    Jin Otsuka & Tsuneo Chonan, Tokyo Med. and Dent. Univ.

14. Ontological studies on myopia
Tutomu Sato et al., Juntendo Univ.
15. Study on presbyopia
Chizuko Teramoto, Nagoya City Univ.
16. Studies on accommodation of the eye in gynecology and obsterics
Yoshio Ohara, Nagoya City Univ.
17. Clinical observation of human semilunar plica
Yasu Komura, Tokyo Jikeikai Med. College
18. Studies on the follicles of the conjunctiva
Takeshi Asakage et al., Nippon Univ.
19. Studies on conjunctival follicles
Nobuhiko Matuo, Okayama Univ.
20. Experimental studies on the difference and similarity between trachoma follicles and nontrachomatous follicles
Kazuo Henmi, Gunma Univ.
21. Inoculation of the trachoma to incubated eggs through many generations and serological studies
Tsurue Jinnai, Hokkaido Univ.
22. Serial passage of trachoma virus using chorioallantoic membrane of fertile chick egg and electron microscopic study
Hidehisa Fujiyama, Hokkaido Univ.
23. Pathohistological studies on pterigium
Tomin Maeda, Gunma Univ.
24. Experimental keratoconjunctivitis caused by the pollen
Sachiko Sugimoto, Nippon Med. College
25. Studies on permiability of the rabbits cornea to sodium ions
Kiyoshi Kurosawa, Univ. of Tokyo
26. Fructuation on corneal fat of the human eye with age and experimental studies on the process of the appearance of fat
Yoshio Kaneko, Nippon Med. College
27. Histochemical studies on the corneal epithelium in vitamin B_2 deficiency
Kenshi Takahashi, Hirosaki Univ.
28. Histological studies on the transplantation of hetero-cornea
Kazuo Hirakawa, Keio Univ.
29. Serological studies on corneal heterografts
Takako Arai, Tokyo Med. College
30. Clinical evaluation of precipitin test in the postoperative course of keratoplasty
Jun Tsutsui, Okayama Univ.
31. Biomicroscopic and histologic findings on the experimental herpetic keratitis
Shusaku Kitano et al., Nippon Univ.
32. Studies on Behçet's disease
Tsutomu Asaoka, Tohoku Univ.
33. Bacteriological investigations of Behçet's syndrome
Hiroshi Ujihara, Univ. of Tokyo
34. Experimental studies on the pathologic retinal change
Shigeru Sonoda, Univ. of Kagoshima
35. Studies on the experimental degeneration of the retina
Osamu Nakagawa, Juntendo Univ.
36. Study on scotopic luminosity curve of degeneratio pigmentosa retinea
Iwao Hara, Kanazawa Univ.
37. Etiology and treatment of experimental degeneration of the retina

based on the hypothesis of blood retinal barrier
Katsuyoshi Mizuno, Nagoya Univ.

38. Studies on the process of light adaptation of the frog retina by short period light beam interception method
Reizo Manabe et al., Osaka Univ.

39. Studies on the relationship between ERG and retinal circuration
Shigesue Onodera, Toho Univ.

40. Experimental studies on the influence of thyroid hormone and thyroid stimurating hormone on the ERG
Naota Kato, Kyoto Univ.

41. Analysis of retinal process by means of electrical phosphenes produced by A.C. sine wave stimuration
Rihei Takahashi, Iwate Med. College

42. Application of flicker-test for pratical use in ophthalmological clinic
Kazuhiro Tokuyama, Yamaguchi Med. School

43. Effect of stress on regeneration of the visual purple of toards
Kikuko Suyama, Osaka City Univ.

44. Experimental studies on influences of antibiotics for retinal metabolism
Yusaburo Kumoi, Mie Univ.

45. Study of the effects of indispensable amino acids on the metabolism of the retina
Tifumi Watanabe, Osaka Med. College

46. Lactic acid in the retina
Yasukazu Kataguchi, Kanazawa Univ.

47. TCA-cycle and citric acid in the retina
Tatsuji Yoshino, Shinshu Univ.

48. Studies on the effect of pantothenic acid on the retina
Kazuo Matsushita et al., Osaka Municipal Transportation Bureau Hospital

49. Study on the Takayasu-Onishi's disease
Kinnosuke Hirose & Harumi Baba, Nagasaki Univ.

50. Genetico-epidemiological study on the occurrence of retinoblastoma in a Japanese populaation
Hiroichi Ogyu, Sapporo Med. College

51. Slit lamp microscopic obserbation of posterior detachment of the vitreous in cases with spontaneous retinal detachment
Toshiaki Takeda, Hiroshima Univ.

52. Studies on fundus findings in hypertention and the function of kidney and liver
Kiyoshi Ishikawa & Dosetsu Kanaizuka, Chiba Univ.

53. Retinal central arterial pressure in arterioscrelotic hypertention
Toshio Suzuki, Niigata Univ.

54. Studies on experimental arterioscrelosis, ocular blood pressure and adrenaline dehydrogenase in the wall of intraoptic central artery
Jihei Kinugasa et al., Osaka Univ.

55. Studies on retinal central arterial pressure and corneal pulse wave of experimental diabetic rabbits
Akira Hagai, Keio Univ.

56. Histological studies on the development of narrow angle glaucoma
Hiroshi Ikui & Shinobu Iwaki, Kurume Univ.

57. Variation of aqueous pressure caused by increase of vitreous pressure
Kazuyuki Kihara, Kumamoto Univ.

58. A Convex plunger of the tonometer
Keiu Suda & Masaharu Fukushima, Kumamoto Univ.
59. Tonographic studies on the influence of the sympathetic nervous system regarding the reguration of the intra-ocular pressure in rabbit
Itsuya Ueno, Kyoto Univ.
60. Relation between glaucoma and blood-sugar content with special reference to the function of mesencephalon
Hiroshi Saito, Chiba Univ.
61. Effect of posterior pituitary hormones on the eye of rabbits
Yukitsura Tanabe, Osaka City Univ.
62. Intraocular pressure of interstitial keratitis
Masazumi Sano, Gifu Pref. Med. College
63. Studies on allergic reaction caused by lens substances
Takashi Mizukawa et al., Osaka Univ.
64. Antigenic structure of cristalline lens
Shinkichi Kizu, Osaka Univ.
65. Polarized light studies of the crystalline lens
Nobuhiro Okada, Univ. of Tokyo
66. Studies on the experimental cataract
Shinji Kurimoto, Tottori Univ.
67. Studies on senile cataract: Anthranilic acid metabolism in senile cataract patients
Wakayama Pref. Med. College
68. Influence of low atmosphere pressure on lens epithelium
Yoshihiro Takagi et al., Tokushima Univ.
69. Studies on early changes in epithelial cells of lens-capsule and ciliary body at experimental cataracts
Takao Okamoto, Tottori Univ.
70. Electron microscopic studies of the ciliary epithelium
Akira Urayama et al., Tohoku Univ.
71. Electron microscopic studies on the fine structure of ciliary epithelium in the rabbit
Hayao Ono, Kurume Univ.
72. Electron microscopy of pigment granules of retinal epithelium
Yoshiaki Taniguch, Kyushu Univ.
73. Electron microscopic observation of the optic nerve
Yoshiaki Yuri, Kobe Med, College
74. Electron microscopic study on lacrimal gland of normal rabbit
Takao Obayashi, Krume Univ.
75. Influence of low frequency wave on the eye
Hiromasa Kishida, Tokyo Jikei-kai School of Med.
76. Circulatory dynamics of peripheral arteries of the head, eye and limb by ultrasonic method
Isaku Suzuki & Shigeo Satomura
Osaka Prefectural Hospital
77. Experimental studies on the coagulation of the aqueous
Toshiyuki Suzuki, Tokyo Med. College
78. Phosphorous metabolism of the aqueous humor of the human
Hiroshi Senoo, Iwate Med. College
79. Experimental studie on ocular manifestation of pantothenic acid deficiency
Aishi Tomizawa, Hirosaki Univ.

80. Effects of antibiotics on riboflavin
Ayako Oike, Nagoya Univ.
81. Fluctuation of glycolysis on secondary degeneration of optic nerve of the dog
Jo Imachi et al., Kobe Med. College
82. Histochemical study on penetretion of lead into the eye
Nobuhiro Kobayashi, Tohoku Univ.
83. Experimental study on diagnogis of orbital diseases
Akira Aoike, Univ. of Tokyo
84. Studies on the determination of position of intraocular foreign body
Masaru Kosaki et al., Osaka Med. Dollege
85. Study on enzymatic zonulysis using α-chymotrypsin
Naohiko Tanaka, Yokohama Univ.
86. Studies on cataract surgery
Takao Dodo, Kyoto Pref. Med. Univ.
87. Studies on the ophthalmic use of Kanamycin
Masao Oishi, Niigata Univ.
88. Studies on the ophthalmic use of Sigmamycin
Tsuneo Ishida, Niigata Univ.
89. Precipicin reaction in agar for studies of EKC
Chie Tanaka et al., Tokyo Women's Med. Dollege
90. Study of pharyngo-conjunctival fever
Sho Ogata et al., Kumamoto Univ.

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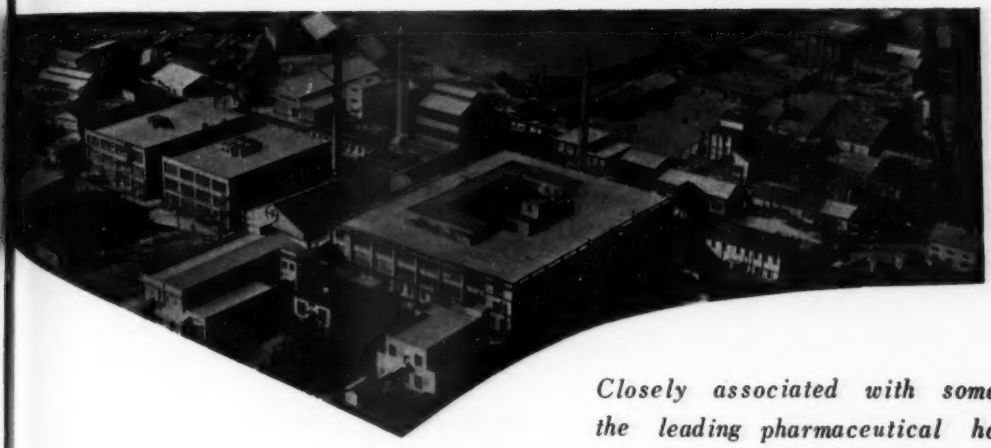

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